

Université de Montréal

**Adaptation florale aux pollinisateurs : étude des Gesneriaceae
antillaises et de l'Impatiente du Cap**

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Étude de l'adaptation florale aux pollinisateurs : les cas des Gesneriaceae Antillaises et de l'Impatiente du Cap

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Résumé

L'environnement a un effet considérable sur les végétaux par différents facteurs abiotiques (climat, sol, urbanisation) ou biotiques (pollinisateurs, herbivores). Les fortes pressions de sélection exercées par ces facteurs sur certains traits phénotypiques aboutissent souvent à des adaptations chez les plantes. Les pollinisateurs exercent une pression de sélection sur les traits floraux qui résultent en des adaptations convergentes que l'on appelle syndromes de pollinisation. L'étude de ces syndromes, mais aussi de la performance de pollinisation de chaque visiteur, permet de mieux comprendre l'intensité de cette pression de sélection ainsi que l'évolution florale.

Dans cette thèse, nous nous sommes appuyés sur l'utilisation de deux modèles d'études végétaux : la famille des Gesneriaceae des Antilles et l'Impatiens du Cap. Ces deux modèles, de par leurs stratégies de pollinisation et leurs variations florales, sont appropriés pour étudier l'adaptation florale aux pollinisateurs. Nous avons ainsi pu tester 1) si la forme florale est expliquée par la forme du bec des colibris pollinisateurs chez les Gesneriaceae antillaises ; 2) si l'espèce *Rhytidophyllum bicolor* Urb. est généraliste en pollinisation et si ses différents types fonctionnels de pollinisateurs ont une performance de pollinisation similaire ; 3) si l'urbanisation affecte la forme florale de l'espèce *Impatiens capensis* Meerb., à travers des changements dans les communautés de pollinisateurs.

Pour tester ces hypothèses, des approches de morphométries géométriques ont été utilisées (hypothèses 1 et 3), ainsi que des observations de pollinisation *in situ* et la mesure du taux de visite (hypothèses 2 et 3). La mesure de performance de pollinisation a été réalisée via le comptage des grains de pollen déposés sur le stigmate après chaque visite, et à la mesure du taux de visite. Enfin, des tests statistiques (ANOVA, régressions linéaires) et analyses multivariées (analyses de redondance, analyse en composantes principales) ont été réalisées sur les données obtenues pour chaque étude.

Les résultats de la première étude montrent une corrélation positive entre la longueur de la corolle de fleurs spécialistes aux colibris et la longueur du bec des pollinisateurs. Bien que moins significatifs, les résultats des comparaisons de formes globales, obtenues par application de morphométrie géométrique, indiquent que cette approche est prometteuse pour ce genre d'analyse. Nous avons démontré que la forme florale des fleurs généralistes est impactée par les colibris pollinisateurs, bien que d'une manière différente des spécialistes.

Pour l'hypothèse 2, la prédiction de stratégie de pollinisation de l'espèce *Rhytidophyllum bicolor* a été validée, puisque cette plante est pollinisée par des colibris, chauves-souris et abeilles. Cependant, face au déclin des populations de colibris après le passage de l'ouragan Matthew sur Haïti, seules les performances de pollinisation des abeilles et des chauves-souris ont pu être mesurées. Les résultats montrent que les chauves-souris sont des pollinisateurs efficaces et conséquents, bien que la performance des abeilles ne soit pas négligeable. Il a ainsi pu être mis de l'avant que la stratégie de pollinisation généraliste semble être un avantage pour les plantes présentes dans les zones sujettes aux fluctuations de populations de pollinisateurs, comme cela peut souvent être le cas sur les îles à la suite du passage d'un ouragan.

La troisième étude montre que l'urbanisation n'a pas d'effet direct sur la forme florale de l'Impatiante du Cap, mais qu'elle a des effets indirects significatifs via les changements causés sur les communautés de pollinisateurs. Sur les six sites échantillonnées les pollinisateurs principaux, *Bombus sp.* Latreille et *Apis mellifera* Linnaeus, sont les mêmes. Cependant les taux de diversité obtenus montrent une variation entre les sites, due à la présence de certaines espèces de pollinisateurs occasionnels dans certains sites et pas dans d'autres. Ces taux ne sont pas plus faibles dans les sites les plus urbains. Les résultats indiquent que certaines formes florales sont associées à des espèces de pollinisateurs particulières. Les différentes espèces de pollinisateurs ayant une pression de sélection différente sur les traits floraux, l'urbanisation a ainsi un impact indirect sur la forme florale chez l'espèce étudiée.

À travers trois études différentes, cette thèse a mis en avant l'impact que l'environnement peut avoir sur les traits floraux, de manière indirecte, via les pollinisateurs. Alors que la deuxième et troisième étude ont montré la pression exercée par les pollinisateurs sur les traits floraux dans différents cas de perturbations de l'habitat, la première étude a permis de mieux comprendre l'adaptation remarquable des fleurs à leurs pollinisateurs, même pour des espèces généralistes en pollinisation.

Mots clés

Traits floraux, Gesneriaceae, Performance de pollinisation, Impatiens, Morphométrie géométrique, Urbanisation, Pollinisateurs

Abstract

The environment has a considerable effect on plants through various abiotic (climate, soil, urbanization) or biotic (pollinators, herbivores) factors. The strong selection pressures exerted by these factors on phenotypic traits often results in adaptations. Pollinators exert selection pressure on floral traits that result in converging adaptations called pollination syndromes. The study of syndromes, as well as the pollination performance of each floral visitor, allows us to better understand the intensity of a/biotic selection pressure and floral evolution.

In this thesis, we relied on two plant models: the Gesneriaceae family in the Antilles, and the common Jewelweed, *Impatiens capensis* Meerb. Due to their pollination strategies and their floral variation, both of these models are suitable for studying floral adaptation to biotic factors, specifically, their pollinators. We were thus able to test the following hypotheses: 1) whether the floral form is explained by the beak shape of pollinating hummingbirds in West Indies Gesneriaceae; 2) whether the Gesneriaceae *Rhytidophyllum bicolor* Urb. has a generalist strategy for pollination and whether their different functional types of pollinators have similar pollination performances; 3) whether urbanization affects the floral form of the common Jewelweed, through changes in pollinator communities.

To test these hypotheses, geometric morphometric approaches were used (hypotheses 1 and 3), as well as *in situ* pollination observations, and estimation of the visitation rate (hypotheses 2 and 3). Pollination performance was measured by counting pollen grains deposited on the stigma after each visit, and by measuring the visitation rate. Finally, statistical tests (ANOVA, linear regressions) and multivariate analyses (redundancy analysis, principal component analysis) were carried out on the data obtained to test each hypothesis.

The first results show a positive correlation between the length of the corolla of flowers specialized for hummingbirds and the beak length of pollinators. Although less significant, our results of the comparisons of global shapes, obtained by applying geometric morphometry, indicate that this approach is promising for this kind of analysis. We show that the floral form of generalist flowers is impacted by pollinating hummingbirds, albeit in a different way from specialists.

For the second hypothesis, our prediction that *R. bicolor* has a generalist pollination strategy was validated, since this plant is pollinated by hummingbirds, bats and bees. However, faced with the decline in hummingbird populations after Hurricane Matthew hit Haiti in 2016, only the pollination performance of bees and bats could be measured. Our results show that bats are efficient and

consistent pollinators of *R. bicolor*, although the performance of bees is not negligible. Thus, it has been possible to put forward that the generalist pollination strategy seems to be an advantage for plants present in areas subject to fluctuations in their pollinator populations, as can often be the case on hurricane-prone islands.

The third study shows that urbanization does not have a direct effect on the flower form of common Jewelweed, but that urbanization does have significant indirect effects through changes caused on pollinator communities. At the six sites sampled, the main pollinators, *Bombus* sp. Latreille and *Apis mellifera* Linnaeus, are the same. However, diversity rates show variation between sites, due to the presence of certain species of occasional pollinators in some sites and not in others. These rates are not lower in the most urban sites. Our results indicate that certain floral forms are associated with particular pollinator species. Since different pollinator species have different selection pressures on floral traits, urbanization has an indirect impact on the floral form in the species studied.

Through three different studies, this thesis highlighted the impact that the environment can have on floral traits, indirectly, via pollinators. While the second and third studies showed the pressure exerted by pollinators on floral traits in different cases of habitat disturbance, the first study helped to better highlight the remarkable adaptation of flowers to their pollinators, even for generalist species in pollination.

Key words

Floral traits, Gesneriaceae, Pollination performance, Impatiens, Geometric morphometry, Urbanization, Pollinators

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Liste des sigles et abréviations

C : Cuba

GBIF: Global Biodiversity Information

H: Hispaniola

J: Jamaica

LA: Lesser Antilles

MEPP: Most Effective Pollinator Principle

NDVI: Normalized Vegetation Index

NY: New York Botanical Garden Herbarium

PC : Principal Component

PCA : Principal Component Analysis

PR : Puerto Rico

RDA : Redundancy Analysis

SU-RSF : population d'Impatientes du Cap du Rang St-François à Laval, en milieu sub-urbain.

SU-RB : population d'Impatientes du Cap de la rue Bergeron à Laval, en milieu sub-urbain.

SU-UFO : population d'Impatientes du Cap dans le bois proche du Golf UFO à Laval, en milieu sub-urbain.

N-RDN : Population d'Impatientes du Cap située dans le Parc Régional de la Rivière-du-Nord, en milieu naturel.

N-OKA : Population d'Impatientes du Cap situé dans le Parc d'OKA, en milieu naturel.

N-SBL : Population d'Impatientes du Cap qui se trouve proche de la limite de la Station de Biologie des Laurentides, en milieu naturel.

« Quand on cherche, on trouve généralement quelque chose, mais ce n'est pas toujours exactement ce qu'on voulait. »

Bilbo le Hobbit – J. R. R. Tolkien (1937)

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Chapitre 1 : Introduction

Une grande partie de la pollinisation des angiospermes est effectuée par les animaux (87.5%) (Ollerton *et al.*, 2011). En choisissant certaines plantes, les pollinisateurs engendrent une pression de sélection qui tend à favoriser des traits spécifiques au niveau des organes reproducteurs, la fleur. Ces traits sont en lien avec la morphologie du pollinisateur et facilitent la pollinisation (Armbruster, 2014; Cresswell, 1998). Ces pressions de sélection seraient responsables de l'évolution de traits similaires qui surviennent indépendamment chez des espèces pollinisées par les mêmes pollinisateurs, tels que les Gesneriaceae des Antilles ou les Lobelioideae des Andes qui exhibent des traits floraux très semblables face à la pollinisation par les colibris ou les chauves-souris (Lagomarsino *et al.*, 2017; Martén-Rodríguez & Fenster, 2008). Cet ensemble de traits est alors nommé stratégie ou syndrome de pollinisation (Fenster *et al.*, 2004). Selon Darwin (1862), l'évolution de la diversité florale est la résultante de la sélection par les pollinisateurs. Si d'autres pressions de sélection existent (e.g. climat, altitude, florivorie, isolation génétique), cette théorie est appuyée par Grant & Grant (1965) qui considèrent que les pollinisateurs jouent un rôle décisif dans la diversification des fleurs. Bien que l'idée soit débattue (Ollerton *et al.*, 2009), cette spécificité de pollinisation continue de faire l'objet de nombreuses études, qui cherchent à mieux comprendre son intérêt et ses conséquences sur la plante.

L'étude de l'évolution des systèmes de reproduction montre que les transitions des systèmes de pollinisation sont survenus selon différents schémas, du type généralisé vers le spécialisé (Chase & Hills, 1992) comme du type spécialisé vers le généralisé (Armbruster & Baldwin, 1998). Certaines plantes sont susceptibles d'être « super généralistes », et ce en particulier dans les régions tempérées, mais aussi dans les régions tropicales (Ollerton & Cranmer, 2002; Ollerton *et al.*, 2006). Les îles des archipels océaniques présentent une faune de pollinisateurs moins riche et un ratio pollinisateur/plante bas par rapport au continent (Traveset *et al.*, 2015), ce qui favorise certaines stratégies de reproduction, telles que la généralisation, la pollinisation par le vent ou l'autopollinisation (Barrett *et al.*, 1996; Martén-Rodríguez *et al.*, 2010). Les plantes insulaires sont donc de bons modèles d'étude de l'évolution des systèmes de pollinisation (Martén-Rodríguez & Fenster, 2008).

1.1. Généralisation et spécialisation

Au sens large en écologie, un individu généraliste peut être représenté comme un consommateur ayant la même force de relation entre toutes les ressources du milieu (Poisot *et al.*, 2012). Par opposition, la spécialisation écologique est le processus par lequel un organisme utilise un ensemble très restreint d'habitats ou de ressources. Un organisme est spécialiste lorsque ce processus adaptatif et écologique l'a mené à utiliser un ensemble réduit de ressources. Plusieurs indices ont d'ailleurs été proposés pour mesurer la généralisation (ou la spécialisation) et la plupart reposent sur (1) la diversité des ressources et (2) les liens avec ces ressources (Poisot *et al.*, 2012).

Lorsqu'on se place au niveau de la pollinisation, la spécialisation est définie par Armbruster & Baldwin (1998) comme une pollinisation fructueuse par un petit nombre d'espèces de pollinisateurs pour une plante donnée. La généralisation est au contraire l'utilisation de plusieurs espèces de pollinisateurs sur une seule même plante, et la visite de plusieurs espèces de plantes pour un même pollinisateur (Waser *et al.*, 1996). Certains considèrent aussi des aspects taxonomiques dans cette définition, et la limite entre spécialisation et généralisation n'est pas toujours claire. Par exemple, une plante pollinisée par plusieurs pollinisateurs appartenant à des genres ou familles différentes serait plus généraliste qu'une plante pollinisée par plusieurs pollinisateurs appartenant à la même famille (Gómez & Zamora, 2006; Johnson & Steiner, 2000). La même idée peut d'ailleurs s'appliquer à des groupes fonctionnels de pollinisateurs (Gómez & Zamora, 2006). On parle de groupe fonctionnel pour un groupe rassemblant des pollinisateurs présentant des caractéristiques ou une morphologie semblable, et une approche similaire de pollinisation, tels que les colibris et les nectariniidés avec la forme de leur bec. Dans certains cas, lorsque les auteurs parlent de spécialisation, d'autres argumenteraient qu'il s'agit de généralistes écologiques. Par exemple certaines fleurs spécialisées vont présenter des traits associés à la pollinisation par les colibris, mais elles sont souvent visitées par plusieurs espèces de colibris, et sont donc à un certain degré des généralistes écologiques (Ollerton *et al.*, 2007).

A travers les différents chapitres de cette thèse, nous utilisons le terme « spécialiste » lorsqu'une plante est pollinisée par un seul type fonctionnel de pollinisateur, par exemple les colibris. Une fleur de plante spécialiste aux colibris peut cependant être pollinisée par plusieurs espèces de colibris. Le terme « généraliste » est ici utilisé pour une plante dont les fleurs sont pollinisées par une gamme de pollinisateurs appartenant à des groupes d'animaux différents, par exemple à la fois les colibris, les chauves-souris et les insectes.

1.2. Adaptation des traits floraux

Depuis de nombreuses années, certains auteurs se sont accordés sur le fait que les traits floraux d'une plante semblent s'adapter en fonction des pollinisateurs qui viennent la visiter (Baker, 1961; Faegri; Grant & Grant, 1965; Herrera, 1996; Stebbins, 1970; Waser *et al.*, 1996). En effet la sélection naturelle favoriserait fréquemment la spécialisation des plantes pour la pollinisation par des animaux spécifiques (P. Aigner, 2005), bien qu'il existe une pression de sélection de la part des pollinisateurs autant chez les plantes spécialistes en pollinisation que chez les généralistes (Gómez *et al.*, 2015; Gómez *et al.*, 2016). Cette pression de sélection persistante sur les traits floraux permet d'assurer la reproduction de la plante, car elle améliore l'attraction, le prélèvement et la déposition de pollen par les pollinisateurs (Armbruster, 2014; Cresswell, 1998). Certains auteurs évoquent même des phénomènes de coévolution entre les plantes et les pollinisateurs, comme le cas des espèces du genre *Heliconia* L. et d'*Eulampis jugularis* Linnaeus (Temeles *et al.*, 2005), ou encore des papillons de nuit chez les *Yuccas* (Baker, 1961). Cependant ces exemples étant bien particuliers, ce phénomène ne fait pas l'unanimité (Ollerton *et al.*, 2009; Waser, 2006).

Pour éviter les erreurs d'interprétation, les observations de pollinisation sur le terrain sont la manière d'éviter les mauvaises prédictions de syndromes de pollinisation (Johnson & Steiner, 2000; Ollerton *et al.*, 2009). Cependant il existe une liste d'adaptations bien connues (Fenster *et al.*, 2004; Rosas-Guerrero *et al.*, 2014) qui permettent une identification assez efficace de la stratégie de pollinisation. La pression de sélection exercée par un type de pollinisation particulier sur différentes familles de plantes amène à l'apparition de traits floraux spécifiques adaptés à un type de pollinisateur dans différentes lignées évolutives indépendantes, qu'on appelle convergence florale. Il existe des exemples bien connus de résultats d'adaptation à des pollinisateurs spécifiques. Les fleurs tubulaires rouges ou jaunes sont adaptés à la pollinisation par les colibris (Abrahamczyk & Kessler, 2015; Castellanos *et al.*, 2004), ceux-ci se nourrissant à l'aube et au coucher du soleil, heures où ces couleurs ressortent le plus au milieu de la végétation (Baker, 1961). Les fleurs en forme de cloche, claires et présentes sur des inflorescences situées sur des branches longues et pendantes accessibles aux pollinisateurs volants, sont adaptées à la pollinisation par les chauves-souris (Baker, 1961). Enfin, la présence d'un éperon de nectar très long témoigne d'une adaptation à la pollinisation par les sphingidés (Baker, 1961; Darwin, 1862; Kaczorowski *et al.*, 2012).

Ainsi la grande diversité de couleurs et de formes de fleurs peut être en grande partie expliquée par la présence de divers pollinisateurs. L'association entre les traits floraux et la forme des pièces buccales des pollinisateurs a été largement étudiée (Agosta & Janzen, 2005; Dalsgaard *et al.*, 2009; Sonne *et al.*, 2020; Temeles & Kress, 2003; Van der Niet *et al.*, 2014), mais c'est la stratégie de pollinisation spécialisée aux colibris qui présente un des meilleurs exemples de convergence florale (Rosas-Guerrero *et al.*, 2014). Ainsi, plusieurs études ont montré une forte corrélation entre la longueur du bec des colibris et la longueur du tube floral des fleurs qu'ils visitent (Cotton, 1998; Dalsgaard *et al.*, 2009; del Coro Arizmendi & Ornelas, 1990; Maglianesi *et al.*, 2014; Nattero & Cocucci, 2007). En exerçant une certaine pression de sélection sur les plantes, les pollinisateurs induisent donc souvent une adaptation forte, qui entraîne des radiations adaptatives dans les familles végétales.

Alors que pour une plante donnée il peut être plus efficace de se spécialiser à un seul type de pollinisateur présentant une grande population et permettant un bon succès reproductif (bien que ce ne soit pas toujours la meilleure stratégie, ceux-ci représentant une ressource variable), pour le pollinisateur au contraire il paraîtrait pour le moins risqué de se spécialiser à une seule espèce de fleur, qui pourrait être une ressource peu fiable, de par sa variabilité temporelle et spatiale (Waser, 2006). On remarque donc que dans la plupart des cas, bien qu'une plante soit très spécialiste d'un pollinisateur, et même si celui-ci semble l'être pour la plante, il va aller visiter un certain panel de plantes afin d'avoir le plus grand nombre de ressources (Waser, 2006). C'est le cas notamment des colibris, qui bien que visitant communément les fleurs ayant des traits associés à une pollinisation ornithophile, peuvent visiter de nombreuses fleurs qui sont en général pollinisées par des insectes, ou des chauves-souris (Abrahamczyk & Kessler, 2010, 2015; Araujo & Sazima, 2003).

1.3. Performance de pollinisation

La présence de traits morphologiques spécialisés chez une plante reflète souvent la présence d'un pollinisateur plus efficace que les autres (Martén-Rodríguez *et al.*, 2010; Stebbins, 1970). Cependant, le pollinisateur le plus performant n'est pas toujours le plus fréquent (Fumero-Cabán & Meléndez-Ackerman, 2007; Mayfield *et al.*, 2001; Niemirski & Zych, 2011; Sahli & Conner, 2007; Zych, 2007). Il est donc nécessaire de mesurer la performance de pollinisation de chaque pollinisateur qui est observé visitant une fleur. L'étude de la performance de pollinisation est d'autant plus essentielle chez les plantes à stratégie de reproduction généraliste, puisque plusieurs pollinisateurs différents contribuent à sa reproduction. Dans la littérature, on retrouve différentes

définitions de la performance du pollinisateur, l'intensité de pollinisation ou l'efficacité du pollinisateur. Ces termes sont souvent présentés selon diverses mesures. De manière générale, la performance est calculée en mesurant la capacité de transfert du pollen par le pollinisateur et son dépôt sur le stigmate. Freitas (2013) définit la performance (appelée efficacité) du pollinisateur comme suit:

$$\text{Performance} = \text{compétence du pollinisateur (composante mâle ou femelle)} \times \text{Taux de visite.}$$

La performance du pollinisateur est la contribution totale du pollinisateur au succès reproductif de la plante, reflétant le produit de la compétence du pollinisateur (individu, population d'une espèce de pollinisateur, ou groupe fonctionnel de pollinisateur), et du taux de visite. Mécaniquement parlant, la compétence du pollinisateur est sa capacité à effectuer le transfert du pollen depuis les étamines vers le stigmate, résultant en la fécondation. Elle est estimée par la quantité de pollen prélevé (composante mâle) ou déposé, le nombre d'ovules fécondés, ou encore le nombre de fruits ou de graines obtenus (composante femelle). Cette compétence est indépendante du taux de visite, mais dépendante des caractéristiques de la population de l'espèce de plante étudiée et des interactions avec les autres espèces de visiteurs (Freitas, 2013). Le taux de visite d'un pollinisateur est exprimé par le nombre d'événements de pollinisation indépendants réalisés par un pollinisateur, mesuré par le nombre de visites par unité de temps (Freitas, 2013). Cette mesure est influencée par la disponibilité des ressources au niveau spatiotemporel, l'assemblage des visiteurs floraux locaux, la densité de la plante étudiée, et l'existence de fleurs compétitives.

Selon Armbruster (2014), la performance de pollinisation (aussi appelée efficacité) est définie d'après la formule suivante :

$$\text{Performance} = \text{Taux de visite} \times \text{Probabilité de contact avec les anthères} \times \text{Probabilité de contact avec le stigmate}$$

En comparaison de la performance de Freitas (2013), celle définie par Armbruster (2014) demande d'évaluer les composantes mâles et femelles dans un même indice.

Enfin, d'après l'étude de Ne'eman *et al.* (2010) la performance est divisée en deux concepts de performance du pollinisateur, que sont le succès de pollinisation et le succès reproductif femelle, selon la formule :

$$\text{Performance} = \text{Succès reproductif mâle (pollen prélevé)} \times \text{Succès reproducteur femelle (pollen déposé, ovules fécondés, graines)}.$$

Le succès reproductif mâle se traduit ici par le dépôt de pollen sur le stigmate, et se mesure par le nombre de grains de pollen prélevés. Le succès reproductif femelle mesure la contribution du pollinisateur à l'ensemble des graines produites suite au dépôt de pollen. Nous utilisons dans cette thèse le terme « performance du pollinisateur », et non « efficacité » qui est utilisée par plusieurs auteurs, pour représenter la contribution du pollinisateur au succès reproductif de la plante, puisque le terme « efficacité » présente trop de définitions diverses.

1.4. Valeur sélective de la plante

Afin de mieux comprendre l'adaptation des plantes à un ou plusieurs pollinisateurs, il est nécessaire de comprendre l'impact de la performance d'un pollinisateur sur la valeur sélective globale de la plante, qui dépend de tous les pollinisateurs possibles. La valeur sélective d'une plante est définie comme sa capacité à transmettre ses gènes aux générations futures. Ici on s'intéresse seulement à la partie de la valeur sélective qui est la résultante de la fonction de pollinisation, ou du service de pollinisation (Aigner, 2001).

Selon Stebbins (1970), une plante est spécialisée pour le pollinisateur qui sera le plus efficace dans sa pollinisation (meilleur dépôt de pollen, meilleur rendement) et le plus abondant : c'est le principe du pollinisateur le plus efficace (Most Effective Pollinator Principle, MEPP). Ce concept a cependant été réfuté depuis, notamment par les travaux d'Aigner (2001). Dans son étude de 2001, Aigner propose un modèle simple à deux pollinisateurs pour prédire quel phénotype sera favorisé et qui peut aider à prédire quand une plante va devenir spécialiste ou généraliste (phénotype adapté aux deux pollinisateurs; Figure 1 A, B). Pour prédire la fonction de pollinisation (= service de pollinisation = valeur sélective de la plante), Aigner propose une équation qui mesure la valeur sélective, en incluant les contributions de chaque pollinisateur dans la pollinisation, mais aussi les interactions entre les pollinisateurs (compromis évolutifs). En effet, la valeur sélective dépend de l'asymétrie des compromis évolutifs de la valeur sélective associés à un pollinisateur particulier.

Quand les compromis évolutifs sont fortement asymétriques, le phénotype de la plante peut subir des changements pour se spécialiser vers des pollinisateurs moins efficaces. Dans le cas d'un visiteur voleur de pollen, ce voleur a une interaction négative sur le pollinisateur plus efficace puisqu'il laissera peu de pollen pour un pollinisateur plus efficace. La forme de la fleur avec la valeur sélective la plus élevée pourrait alors être celle correspondant au voleur.

Selon Aigner (2001, 2006) les fleurs sont souvent visitées par un assemblage taxonomique très divers de pollinisateurs potentiels, en plus de ceux pour lesquels elles semblent spécialisées. Cet assemblage variant dans le temps, les pollinisateurs pour lesquels la plante semble le plus spécialisée peuvent être moins efficaces que les pollinisateurs pour lesquels elle n'a pas de spécialisation apparente.

1.5. Les stratégies de pollinisation généralistes

La meilleure explication à l'existence des systèmes de pollinisation généralistes semble être la variabilité spatiotemporelle de l'abondance et de la composition de l'assemblage de pollinisateurs d'une plante (Gómez & Zamora, 2006). Ces assemblages de pollinisateurs peuvent en effet varier au fil des ans, mais aussi de façon saisonnière au cours d'une année (Ashman & Stanton, 1991; Mayfield *et al.*, 2001), ce qui résulte en deux types d'adaptations possibles : la spécialisation ou généralisation à court terme et la spécialisation ou généralisation à long terme (Brosi, 2016). Cette notion de fluctuation temporelle de la communauté de pollinisateurs a été intégrée dans la formule de calcul de la valeur sélective de Waser *et al.* (1996), puisque leur modèle intègre l'abondance, le taux de visite ainsi que l'efficacité de chaque pollinisateur. Ce modèle a ensuite été complexifié afin d'introduire la fluctuation des pollinisateurs dans le temps. Cela change la vision sur le long terme, puisque de cette manière une plante pourrait être notée comme spécialiste une année et généraliste la suivante si on l'observe à des moments différents. On pourrait donc faussement conclure qu'une plante est spécialiste parce qu'elle a été observée sur un court laps de temps. Sur le long terme, Waser *et al.* (1996) démontrent que selon leur modèle, les fluctuations temporelles favorisent le généralisme. Il existe également des cas où la plante est généraliste au niveau de l'espèce, mais sera spécialiste si on se place au niveau de la population ou de la région, dû à l'apparition d'adaptations pour les pollinisateurs locaux suite à des isolements génétiques (Thompson, 1994).

Il se pourrait d'ailleurs que l'état généraliste ne soit pas le cas particulier, mais au contraire souvent le cas le plus commun rencontré chez les plantes (Ollerton *et al.*, 2007; Waser *et al.*, 1996), puisque qu'il est largement répandu dans les systèmes naturels de pollinisation (Armbruster *et al.*, 2000; de Brito *et al.*, 2017; Herrera, 1996; Olesen, 2000). C'est le cas de certaines plantes, telles que certaines espèces des genres *Penstemon*, *Erythrina* ou *Burmeistera*, dont l'apparence porte à penser qu'elles sont spécialisées à la pollinisation par les colibris, mais qui sont également pollinisées par d'autres types de pollinisateurs (Castellanos *et al.*, 2003; Etcheverry *et al.*, 2012; Muchhala & Thomson, 2010).

L'utilisation d'une stratégie généraliste peut se révéler utile pour la survie de la plante, notamment sur les îles où les populations de pollinisateurs sont plus faibles que sur les continents (Olesen & Jordano, 2002) et sujettes aux fluctuations temporelles, comme lors d'ouragans qui dévastent les populations d'oiseaux et causent une limitation de ressources pour les espèces ornithophiles (Rathcke, 2000). Les plantes généralistes vont alors pouvoir être assurées d'une pollinisation même si certains pollinisateurs sont manquants (Mayfield *et al.*, 2001). Les plantes ayant un système de pollinisation généraliste pourraient donc être privilégiées dans un cas d'environnement hautement variable et perturbé (Waser *et al.*, 1996). Dans les Antilles, les plantes généralistes de plaines alluviales pollinisées par les petits colibris et les insectes pourraient être pollinisées moins efficacement que les plantes d'altitudes pollinisées par les colibris de taille plus grande, et ces plantes généralistes seraient également moins touchées par le manque de pollinisateurs (Dalsgaard *et al.*, 2009).

La généralisation dans les systèmes de pollinisation pourrait être favorisée de façon évolutive dans des endroits continentaux perturbés, ouverts et élevés en altitude où la probabilité de présence de pollinisateurs spécialistes est faible (Armbruster & Baldwin, 1998; Martén-Rodríguez *et al.*, 2010). En effet il semblerait que les plantes des communautés tropicales ne présentent pas moins de généralisme que celles des milieux tempérés et des régions subtropicales (Ollerton & Cranmer, 2002; Ollerton *et al.*, 2006). Dans ces environnements, un système de pollinisation généraliste va souvent produire une plus grande quantité de nectar et attirer une grande diversité d'insectes par exemple, augmentant le succès reproductif de la fleur par rapport à un système de pollinisation spécialiste (de Brito *et al.*, 2017). La généralisation serait ainsi une stratégie adéquate pour assurer l'établissement d'espèces de plantes dans des endroits où les pollinisateurs spécialisés sont moins prévisibles (Waser, 2006). La généralisation pourrait également apporter un avantage au niveau

des réseaux trophiques. La généralisation, favorisant l'utilisation d'une large série de pollinisateurs, permettrait une certaine stabilité dans les communautés (Thébault & Fontaine, 2010).

La variation dans les syndromes de pollinisation affecte le potentiel de diversification florale lorsqu'il y a présence d'un compromis évolutif pour obtenir les services de pollinisateurs (Aigner, 2001; 2005). La généralisation de pollinisation peut mener à une diversification lorsque les variations géographiques dans les assemblages de pollinisateurs produisent une mosaïque de phénotypes optimale. La variation géographique n'aurait pas d'effet sur la diversification florale lorsque les échanges plantes-pollinisateurs sont absents, si les phénotypes représentent un équilibre stable qui fonctionne bien avec une variété de pollinisateurs. La variation dans les environnements de pollinisation pourrait amener à de la divergence adaptative si la généralisation est maintenue comme un équilibre instable entre différentes adaptations optimales pour chaque pollinisateur. Bien que la spécialisation permette une bonne performance de pollinisation, la présence de la généralisation serait donc plus avantageuse dans un cas de variation spatiale des pollinisateurs.

1.6. Effet de l'environnement et de la géographie

Le type fonctionnel de pollinisateur semble avoir le plus d'effet sur l'évolution des traits floraux. Cependant, d'autres facteurs, biotiques ou abiotiques, possèdent aussi un effet notable sur les changements de traits floraux. Les changements peuvent faire suite à l'établissement dans une nouvelle région, présentant des facteurs abiotiques nouveaux tels que le climat, ou le sol (Berry & Bjorkman, 1980; Jump & Peñuelas, 2005; Marschner, 1991), comme cela peut être le cas pour les îles (Martén-Rodríguez *et al.*, 2010) ou pour les zones urbaines (King & Buckney, 2000; Neil & Wu, 2006). Pour ce qui est des facteurs biotiques, autre que l'adaptation aux pollinisateurs, on retrouve chez certaines familles de plantes telles que les passiflores une adaptation à se défendre contre l'herbivorie, grâce à divers procédés phénotypiques ou chimiques (Chew & Courtney, 1991; Kessler & Baldwin, 2002; Young *et al.*, 2003).

Dalsgaard *et al.* (2009) démontrent notamment dans leur étude que le degré de spécialisation d'une plante peut varier grandement en fonction de la morphologie du pollinisateur et de l'environnement. Ce dernier semblerait avoir un effet certain sur les interactions plantes-pollinisateurs, autant au niveau des espèces que des communautés, ceux-ci voyant leur richesse spécifique et leur composition varier avec l'altitude, la pluviométrie, le taux d'urbanisation (Bates *et al.*, 2011; Wenzel *et al.*, 2020) ou encore la température (Colles *et al.*, 2009; Dalsgaard *et al.*, 2009). Par

exemple dans les tropiques, un certain nombre de plantes spécialisées à la pollinisation par les grands colibris se trouvent dans des environnements froids et pluvieux, souvent à des hautes altitudes. À l’opposé, les plantes pollinisées par des petits colibris, plus généralistes, poussent dans des environnements secs et chauds, en basse terre (Dalsgaard *et al.*, 2009).

La localisation est aussi une variable à prendre en compte pour la spécialisation ou la généralisation d’une plante. Ainsi les plantes généralistes sont communes sur les îles océaniques (Olesen *et al.*, 2002). La généralisation chez les Gesneriaceae du Nouveau Monde n’existe que sur les îles (Martén-Rodríguez *et al.*, 2015) indiquant une forte influence de la géographie sur la stratégie de pollinisation mise en place en fonction des ressources disponibles. La géographie influence les niches écologiques des fleurs, et en tenant compte des pollinisateurs comme des ressources, va avoir un effet sur leur adaptation à ces ressources plus ou moins présentes et accessibles.

Bien que, pour les plantes, l’étude de la valeur sélective soit primordiale pour comprendre l’impact de l’efficacité des pollinisateurs et l’adaptation qui peut en résulter, la valeur sélective d’un organisme informe également sur d’autres types de pressions. L’environnement dans lequel un individu évolue et les facteurs abiotiques peuvent également impacter sa valeur sélective, et par extension avoir un effet sur son phénotype. L’urbanisation, qui apporte des facteurs abiotiques différents d’un milieu naturel, peut impacter la valeur sélective des êtres vivants qui doivent y évoluer. Il a notamment été montré que la valeur sélective de certaines chauves-souris était différente entre milieux urbain et non urbain, puisque que les populations urbaines présentaient une diminution de la condition corporelle et de la production de juvéniles (Coleman & Barclay, 2011; Russo & Ancillotto, 2015).

1.7. Effet de l’urbanisation

La valeur sélective des plantes et l’adaptation florale qui en résulte est d’autant plus d’actualité dans un contexte d’urbanisation croissante des territoires. L’étalement urbain et l’instauration de terres agricoles, ainsi que les changements climatiques, impactent grandement les plantes indigènes qui se trouvent dans les milieux naturels modifiés (González-Varo *et al.*, 2013; Marschner, 1991; Neil & Wu, 2006). Les changements de qualité de sol, la perte de ressources (McKinney, 2002; Wenzel *et al.*, 2020), la création d’îlots de chaleurs, mais aussi la fragmentation du milieu ont un impact sur les plantes de manière directe, car ces perturbations modifient les caractéristiques de l’habitat (Berry & Bjorkman, 1980; King & Buckney, 2000; Shannon, 1997). Ces dernières années,

un certain nombre d'études se sont penchées sur l'évolution de la faune et la flore en milieu urbain (Johnson & Munshi-South, 2017; Johnson *et al.*, 2015; Rivkin *et al.*, 2019; Santangelo *et al.*, 2018). Pour de nombreuses espèces l'impact majeur est une baisse de la richesse spécifique (Cam *et al.*, 2000; Gortat *et al.*, 2014; Łopucki *et al.*, 2013; Marzluff, 2001). Dans les milieux où le taux d'urbanisation est plus bas, on observe cependant une augmentation de cette richesse, qui peut être en partie expliquée par la présence d'espèces introduites par l'homme (Dolan *et al.*, 2011; McKinney, 2008; Walker *et al.*, 2009).

Dans un milieu urbain, où les facteurs biotiques et abiotiques sont souvent modifiés, il semblerait que seules les espèces à fort potentiel d'acclimatation ou d'évolution puissent s'adapter. En effet, des études menées sur les espèces animales ont montré que certaines espèces sont plus susceptibles de s'adapter à ce nouveau milieu que d'autres (Crocì *et al.*, 2008; Ordeñana *et al.*, 2010; Russo & Ancillotto, 2015). Les espèces indigènes ayant un potentiel adaptatif moins fort sont donc plus menacées d'extinction locale (McKinney, 2002) et doivent se relocaliser dans de nouveaux lieux (Alberti *et al.*, 2017; Stark *et al.*, 2020).

L'urbanisation impacte également les espèces ayant un certain potentiel d'adaptation, puisqu'il a été remarqué des changements biologiques - phénotype, génétique, survie - entre les individus présents en milieu urbain comparativement à ceux présents en milieu naturel (Alberti *et al.*, 2017; Merckx *et al.*, 2018; Russo & Ancillotto, 2015; Schmidt *et al.*, 2020; Turgeon *et al.*, 2015). Cela suggère que l'urbanisation a un effet sur le phénotype des espèces qui évoluent en milieu anthropisé (McKinney, 2008). C'est notamment ce que plusieurs études ont mis en avant dans le cas des plantes : face aux changements qu'apporte l'urbanisation, plusieurs espèces ont vu leur phénotype, leur croissance ou encore leur temps de floraison se modifier (Alberti *et al.*, 2017; Fisogni *et al.*, 2020; Neil & Wu, 2006; Rivkin *et al.*, 2020).

Cependant, l'urbanisation apporte également des impacts indirects pour les espèces végétales, puisque les pollinisateurs sont eux aussi directement impactés par le changement de l'environnement, via la perte de ressources nutritives, la perte d'habitat ou encore l'utilisation de pesticides (Wenzel *et al.*, 2020).

Les espèces pollinisatrices subissent l'effet de changements de paramètres abiotiques, tels que la hausse de température ou les lumières artificielles (Altermatt & Ebert, 2016; Hamblin *et al.*, 2018), les changements de ressources florales (Theodorou *et al.*, 2017), ou encore de la fragmentation d'habitat (Geslin *et al.*, 2013; Soga *et al.*, 2014). L'abondance, la richesse spécifique et le taux de

visite des pollinisateurs seront affectés dans les milieux urbains (Bates *et al.*, 2011; Burdine & McCluney, 2019; Verboven, Uyttenbroeck, *et al.*, 2014), de même que la composition des communautés (Geslin *et al.*, 2013; Martins *et al.*, 2017; Matteson *et al.*, 2013), comparativement aux milieux naturels. Cependant ces changements ne sont pas toujours caractérisés par une diminution de diversité d'espèces ou d'abondance (Martins *et al.*, 2017; Quistberg *et al.*, 2016; Verboven, Uyttenbroeck, *et al.*, 2014) car les habitats à niveau d'urbanisation intermédiaire proposent un niveau de ressources bien supérieur aux habitats agricoles, en lien avec la présence de parcs et de jardins qui fournissent un haut niveau de ressources florales et de potentiel de nidification, avec peu de pesticides et de prédation (Carper *et al.*, 2014; McFrederick & LeBuhn, 2006; Plascencia & Philpott, 2017; Quistberg *et al.*, 2016; Wray & Elle, 2015). Les services de pollinisation seraient même plus efficaces en milieu urbain (Potter & LeBuhn, 2015; Theodorou *et al.*, 2016; Verboven, Aertsens, *et al.*, 2014).

L'urbanisation pouvant amener à un changement dans la communauté de pollinisateurs, cette pression anthropique a alors un impact indirect sur les espèces végétales dont la reproduction se base sur la pollinisation. Les effets directs et indirects de l'urbanisation sur les plantes ont été décrits dans plusieurs études au cours des dernières années (Barker, 2018; Neil *et al.*, 2014; Rivkin *et al.*, 2020; Theodorou *et al.*, 2017; Ushimaru *et al.*, 2014; Wray & Elle, 2015). Face à une communauté de pollinisateurs variés, une plante trop spécialiste pour un pollinisateur disparu pourrait ne plus voir son cycle reproductif complété. Face à ce changement radical, les espèces de plantes généralistes en pollinisation pourraient être plus adéquates pour s'adapter.

1.8. Modèles d'études

1.8.1. Les Gesneriaceae antillaises

Un bon modèle pour l'étude des relations plantes-pollinisateurs et de la spécialisation de pollinisation est la famille des Gesneriaceae dans les Antilles, plus particulièrement les genres *Gesneria* L. et *Rhytidophyllum* Mart. Les Gesneriaceae présentent une grande diversité de formes florales, de couleurs, de parfums et de nectars qui attestent de l'adaptation des plantes à de nombreux pollinisateurs tels que colibris, chauves-souris, abeilles ou papillons de nuit (Perret *et al.*, 2003). On retrouve 67 espèces au sein du genre *Gesneria*, avec une grande variété de formes florales et de croissance (Skog, 1976). Le genre *Rhytidophyllum* présente 23 espèces de plantes arbustives dotées de corolles tubulaires ou en forme de cloche (Martén-Rodríguez *et al.*, 2009;

Skog, 1976). Les espèces présentant des fleurs tubulaires aux couleurs vives, telles que le rouge, sont pollinisées par les colibris (Martén-Rodríguez *et al.*, 2009). Les fleurs en forme de cloche et de couleur verte ou blanche et qui produisent de grandes quantités de nectar pendant la nuit sont pollinisées principalement par les chauves-souris ou les papillons de nuit (Martén-Rodríguez *et al.*, 2009; Sanmartin-Gajardo & Sazima, 2005). D'autres espèces, qui ne se trouvent que sur les îles (Martén-Rodríguez *et al.*, 2015), sont généralistes et attirent plusieurs types de pollinisateurs : les colibris, les chauves-souris et les insectes (cf. Figure 2). Ces plantes vivaces présentent des fleurs protogynes, c'est-à-dire dont les organes femelles sont matures avant les organes mâles (Martén-Rodríguez *et al.*, 2015). Martén-Rodríguez *et al.* (2010) ont démontré que l'ancêtre du genre avait une corolle tubulaire, ce qui laisse supposer que la pollinisation était alors effectuée uniquement par les colibris. Par la suite, il y aurait eu plusieurs évolutions indépendantes vers la pollinisation par les chauves-souris ou la stratégie de pollinisation généraliste, montrant que ces caractères peuvent se transformer fréquemment au cours de l'évolution, ce qui fait de ce groupe un sujet idéal pour étudier l'évolution des systèmes de reproduction.

Bien que ces études permettent d'établir les syndromes de pollinisation de ces genres et leur évolution (Martén-Rodríguez & Fenster, 2008; Martén-Rodríguez *et al.*, 2009; 2010; 2015), certains points restent à élucider, tels que les relations entre ces plantes et leurs pollinisateurs, leur impact sur la valeur sélective de la plante en fonction des différentes formes florales, les événements qui ont occasionné ces changements de syndromes, ou encore les avantages d'être généraliste.

1.8.2. *L'Impatiente du Cap*

Le genre *Impatiens* a été largement étudié pour sa variation florale (Abrahamczyk *et al.*, 2017; Rust, 1977; Travers *et al.*, 2003; Wang *et al.*, 2013; Young, 2008). L'impatiente du Cap *Impatiens capensis* Meerb. est une espèce de plante native d'Amérique du Nord (Rust, 1977). Elle croît près et le long des rivières mais peut aussi être retrouvée en milieu urbain (Barker & Sargent, 2020). Un plant peut atteindre 1,5 mètre de hauteur et présente une saison de végétation allant de mai à juillet, avec une période de floraison allant de juillet à fin septembre (Lu, 2002). Cette espèce possède deux types de fleurs : des fleurs chasmogames (fleurs épanouies pour permettre la pollinisation croisée) dans les populations exposées au soleil ; et des fleurs auto-fertiles cléistogames (fleurs ne s'épanouissant pas à maturité) dans les populations se trouvant sous couvert forestier, sans soleil

direct. Les fleurs chasmogames sont protandres (Rust, 1977), zygomorphes, de couleur jaune-orange avec des tâches rouges et présentent un éperon (Figure 3 A). Le fruit est une capsule à déhiscence explosive.

Les principaux pollinisateurs de l'Impatiente du Cap sont *Bombus sp.*, *Apis mellifera*, *Archilochus colubris* (colibri à gorge rubi) et parfois *Vespula sp.* d'après Rust (1977). Cette plante généraliste en pollinisation, semblant bien s'adapter au milieu urbain, semble un sujet prometteur pour l'étude de l'impact de l'urbanisation sur les fleurs, par les changements de communauté de pollinisateurs. Des résultats préliminaires ont montré une variation significative de la forme florale entre trois populations urbaines de la région de Montréal (Burkiewicz J. et Joly S., non publié), indiquant ainsi que cette espèce pourrait être un bon modèle d'étude de l'effet de l'urbanisation sur la forme florale.

1.9. Objectifs et hypothèses

La problématique globale de mon projet de recherche peut se résumer comme suit : Comment les interactions plante-pollinisateur influencent-elles l'évolution des traits floraux des genres *Gesneria* et *Rhytidophyllum* dans les Antilles, ainsi que d'*Impatiens capensis* dans la région de Montréal ?

Trois hypothèses seront testées dans ce contexte :

- 1) La forme des corolles des fleurs pollinisées par les oiseaux est expliquée par la forme des becs des espèces de colibris qui les pollinisent,
- 2) L'espèce *Rhytidophyllum bicolor* a une stratégie de pollinisation généraliste, selon sa forme florale
- 3) Les communautés de pollinisateurs sont différentes entre milieu urbain et naturel et influencent différemment la forme des fleurs chez l'Impatiente du Cap.

Ces hypothèses peuvent être regroupées en trois thèmes de recherche: l'hypothèse 2 porte sur **la performance des pollinisateurs** et comporte des études sur le terrain, les hypothèses 1 et 3 portent sur **l'adaptation des plantes aux pollinisateurs** et comportent des études morphométriques.

Les syndromes de pollinisation de plusieurs espèces de Gesneriaceae des Antilles ont été bien étudiés (Martén-Rodríguez & Fenster, 2008; Martén-Rodríguez *et al.*, 2009; Martén-Rodríguez *et al.*, 2010; Martén-Rodríguez *et al.*, 2015; Skog, 1976) et leurs pollinisateurs ont été identifiés. Cependant, le degré d'adaptation florale au pollinisateur n'a pas été étudiée dans ce groupe. Puisque

la stratégie de pollinisation spécialisée par les colibris a été largement étudiée chez d'autres groupes (Cotton, 1998; Dalsgaard *et al.*, 2009; Maglianesi *et al.*, 2014; Temeles & Kress, 2003), il semblait intéressant de tester à nouveau cette relation pour un groupe où cela n'avait pas encore été étudié, en y incorporant le syndrome généraliste. La première hypothèse a pour objectif de comparer la forme florale à la forme du bec du colibri pollinisateur, et de mieux connaître l'effet du pollinisateur sur la forme florale des plantes spécialistes et généralistes.

Plusieurs espèces des genres *Rhytidophyllum* et *Gesneria* sont bien documentés quant à leurs traits floraux et pollinisateurs (Martén-Rodríguez & Fenster, 2008; Martén-Rodríguez *et al.*, 2009; 2010; 2015; Skog, 1976). Ce n'est cependant le cas que de 25 des 90 espèces présentes dans les Antilles. De plus, pour les espèces déjà étudiées, la performance de pollinisation n'a encore jamais été mesurée, seul le taux de visite des pollinisateurs ayant été apporté. Afin de mesurer la performance de pollinisation d'une espèce généraliste et de tester l'hypothèse d'Aigner (2001) sur la valeur sélective du phénotype floral chez une espèce généraliste en pollinisation, nous avons choisi d'étudier l'espèce *Rhytidophyllum bicolor* dont les pollinisateurs ne sont pas connus et qui a une morphologie de type généraliste. L'objectif de cette hypothèse est donc d'identifier les pollinisateurs de cette espèce, de mesurer la performance de chaque pollinisateur, et de déterminer l'importance de chacun sur la forme florale.

Enfin l'objectif de la dernière étude est de mesurer l'effet que peut avoir l'urbanisation sur la forme florale, chez une espèce indigène d'Amérique du Nord, présente à la fois en milieu naturel et urbain. En effet, bien que certains effets de l'urbanisation sur les plantes (production de graines, floraison) ont été décrits dans plusieurs études récentes (Barker, 2018; Neil *et al.*, 2014; Rivkin *et al.*, 2020; Theodorou *et al.*, 2017; Ushimaru *et al.*, 2014; Wray & Elle, 2015), nous n'avons pas connaissance d'étude se penchant sur un potentiel effet sur la forme florale. À cause de la possibilité de maladaptation des plantes dans un nouveau milieu et de l'effet connu sur d'autres traits, il semble nécessaire de connaître l'impact de l'urbanisation sur la forme florale des espèces indigènes.

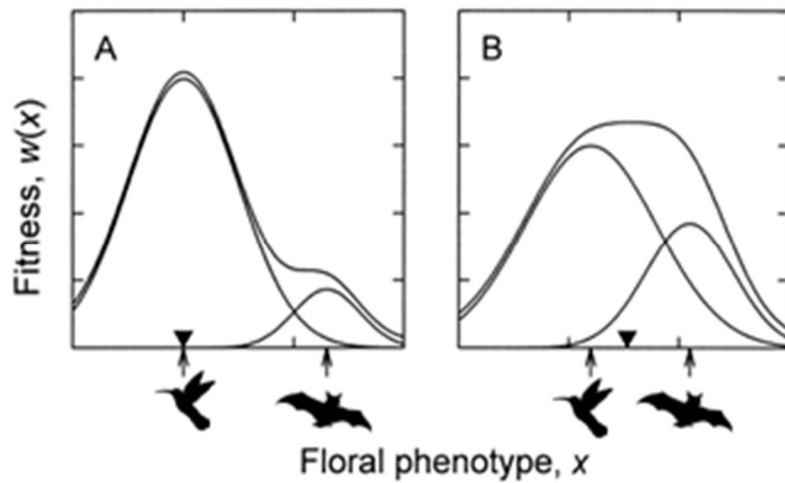


Figure 1. Valeur sélective du phénotype floral (axe des x) en fonction de chaque type de pollinisateur. Lorsque qu'un pollinisateur est beaucoup plus efficace que l'autre (A), le phénotype correspondra au phénotype optimal pour ce pollinisateur. Dans le cas où il n'y a pas de différence significative entre l'efficacité de chaque pollinisateur (B), le phénotype correspondra au point de fitness maximum, qui représente un intermédiaire entre les phénotypes « spécialisés ». Figure adaptée de Aigner (2001)



Figure 2. Les trois formes de fleurs principales des Gesneriaceae antillaises. *Rhytidophyllum rupicola* (Urb.) C.V. Morton est associée au syndrome colibri (A), *Gesneria fruticosa* (L.) Kuntze est pollinisée par des chauve-souris (B) et *Rhytidophyllum auriculatum* Hook. est généraliste (C). Source : Julie Faure



Figure 3. Fleurs chasmogames d'*Impatiens capensis*. Vue de profil (A) et de face (B). Source : Julie Faure

Chapitre 2 : Comparaison de la forme de la corolle et du bec des pollinisateurs chez les Gesnériacées antillaises

Titre original: Does it fit, can I drink? A comparison of Gesneriaceae flower shape and the beak shape of their hummingbird pollinators in the Greater Antilles

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Contribution des auteurs :

Julie Faure et Simon Joly ont conçu l'étude. Julie Faure, John L. Clark et Silvana Marten-Rodriguez ont collecté les données. Julie Faure et Simon Joly ont analysé les données. Julie Faure a écrit la version initiale du manuscrit et tous les auteurs ont contribué à l'écriture de la version finale.

2.1. Abstract

- *Background and Aims:* Pollinators are known to impose strong selection on floral shape. Particularly well studied is the relationship between the flowers of plant species specialized on hummingbird pollination and the beaks of their pollinators. However, most studies to date have evaluated this relationship based solely on length and curvature measurements. Here we use traditional techniques and geometric morphometrics to test for an association between the flower shape of Antillean Gesneriaceae and their hummingbird pollinators. We also tested if the relationship was different for pollination specialists (one functional group of pollinators: hummingbirds) and generalists (more than one functional group of pollinators: hummingbirds, bats and insects).
- *Methods:* We used linear measurements and geometric morphometrics with regression-based analyses (multiple linear regression, redundancy analyses) to test if and how beak shape of hummingbirds explains variation in corolla shape of the Antillean Gesneriaceae they pollinate.
- *Key Results:* We found that variation in corolla length, curvature and overall shape can be explained by different aspects of hummingbird beak shape, but that the relationship differed for pollination specialists and generalists. For instance, a positive association between corolla length and beak length was observed for specialists but not for generalists. Although few statistical tests were significant, the proportion of variance explained was often important (21%-51%), in particular for geometric morphometric approaches.
- *Conclusions:* Our study supports the use of geometric morphometrics for studying the association between flowers and the mouthparts of animals that pollinate them. The correspondence between the shape of corollas and pollinator bills provides support for the idea that pollinator-mediated selection influences the evolution of corolla shape in Antillean gesneriads. However, the strength of the association varied between generalists and specialists suggesting a fundamental difference in selective regimes that warrants further investigation.

2.2. Introduction

Flowering plants experience persistent selection on reproductive traits to ensure sexual reproduction (Cresswell, 1998). For animal pollinated species, selection on floral traits is often mediated by pollinators in traits related to pollinator attraction, pollen removal and pollen deposition (Armbruster *et al.*, 2014). Such selection pressures imposed by specific pollinators often lead to the evolution of similar traits in independent evolutionary lineages—i.e., the concept of pollination syndromes (Faegri & Van Der Pijl, 2013). Many such convergent floral traits are well known (Fenster *et al.*, 2004; Rosas-Guerrero *et al.*, 2014), such as red tubular flowers for hummingbird pollinated plants, or bell-shaped, light colored, nocturnal flowers for bat pollinated plants (Baker, 1961).

The relationship between hummingbirds and hummingbird-pollinated plants has long fascinated botanists and evolutionary biologists (Darwin, 1876) and the hummingbird pollination syndrome has been one of the best supported patterns of floral convergence (Rosas-Guerrero *et al.*, 2014). However, plant-hummingbird interactions are often asymmetric, and most hummingbirds are generalist pollinators that visit several plant species (Abrahamczyk *et al.*, 2015; Dalsgaard *et al.*, 2008; del Coro Arizmendi & Ornelas, 1990; Ollerton *et al.*, 2007; Snow & Snow, 1980). Moreover, while hummingbirds commonly visit plants species with ornithophilous flowers, they can also visit plants primarily pollinated by insects or bats (Abrahamczyk & Kessler, 2010, 2015; Araujo & Sazima, 2003). Conversely, flowers that apparently fit the hummingbird pollination syndrome may also be pollinated by others types of floral visitors (Castellanos *et al.*, 2003; Etcheverry *et al.*, 2012; Muchhala & Thomson, 2010). Interestingly, both theoretical and empirical studies have shown that even minor pollinators have the potential to influence the evolution of floral traits (Aigner, 2001, 2004, 2006; Mayfield *et al.*, 2001). As such, hummingbirds have the potential to influence the floral shape of all the plant species they pollinate, even species that are considered pollination generalists. Finally, flowers specialized on hummingbird pollination may have adaptations to deter other types of pollinators, in addition to traits that facilitate pollination by hummingbirds. One example is the narrow corolla of some hummingbird-pollinated species (Castellanos *et al.*, 2004; Pellmyr, 2002).

The association between floral shape and the mouthparts of their pollinators has been the focus of several recent studies (Agosta & Janzen, 2005; Dalsgaard *et al.*, 2009; Sonne *et al.*, 2020; Temeles

& Kress, 2003; Van der Niet *et al.*, 2014). This association is expected to be important for the mechanical fit, proper pollen export, and pollen deposition. Several studies have shown a strong positive correlation between hummingbird beak length and corolla tube length of the flower they pollinate (Cotton, 1998; Dalsgaard *et al.*, 2009; del Coro Arizmendi & Ornelas, 1990; Maglianesi *et al.*, 2014; Nattero & Cocucci, 2007); however, the relationship is generally not perfect (Cotton, 1998; Maglianesi *et al.*, 2014). As mentioned above, hummingbirds are usually generalists and they often pollinate plants with a broad range of corolla lengths (Araujo & Sazima, 2003; del Coro Arizmendi & Ornelas, 1990). In fact, controlled experiments have shown that all else being equal, hummingbirds prefer feeding on short and strait (i.e., non-curved) corollas (Maglianesi *et al.*, 2015; Temeles *et al.*, 2009). The contrast between such findings obtained in controlled environments and the observed correlations between corolla length and beak length observed in nature (see Maglianesi *et al.*, 2015 for a comparison of experimental and natural conditions) could be explained by niche partitioning. Thus, competition for limited resources could lead to plant-pollinator co-adaptation (Kodric-Brown *et al.*, 1984; Maglianesi *et al.*, 2015; Temeles *et al.*, 2013). In addition, there is evidence of a correlation between corolla length and nectar volume (del Coro Arizmendi & Ornelas, 1990; Kodric-Brown *et al.*, 1984), and of reduced handling times by hummingbirds in flowers that better fit their beaks (Maglianesi *et al.*, 2014). This might also help explain why the correlation between hummingbird beaks and the flowers of species they pollinate is frequently observed in nature.

Beyond strict length comparisons, the fit between the shape of hummingbird beaks and the corollas they pollinate has also attracted much interest (Stiles, 1975; Temeles *et al.*, 2000), although few studies have quantified this relationship in natural communities or within a comparative framework. One exception is the study by Maglianesi *et al.* (2014) that found significant correlations between beak curvature and corolla curvature, as well as between hummingbird body mass and the volume of corollas in three communities of Costa Rica. Several methods are now available to study the overall shape of organisms, such as geometric morphometrics or elliptical Fourier analysis, and they have been used recently to study both bird beak shape evolution (Berns & Adams, 2010; Foster *et al.*, 2008) and corolla shape evolution (Gómez *et al.*, 2016; Joly *et al.*, 2018; Smith & Kriebel, 2018). However, these methods have yet to be used to compare flower and pollinator shapes.

The subtribe Gesneriinae (Gesneriaceae) is a fascinating clade for investigating the relationship between hummingbird bills and floral shape because it represents a monophyletic lineage that diversified in the Caribbean and it exhibits a broad range of corolla shapes. This group of 94 species is mostly pollinated by hummingbirds, either exclusively (functional specialists) or together with bats and insects (mixed-pollination syndromes; functional generalists), although there are also species exclusively pollinated by bats or insects (Martén-Rodríguez *et al.*, 2009; 2015). It is thus of interest to quantify and compare the degree of fit between hummingbird bill shape and corolla shape for pollination specialists and generalists, which we expect to be of lesser importance for the latter, due to trade-offs with other pollinators (Aigner, 2001; Lagomarsino *et al.*, 2017; Martén-Rodríguez *et al.*, 2009; Muchhala, 2007a). Pollinator information in the group is supported by substantial high-quality pollination data (Martén-Rodríguez & Fenster, 2008; Martén-Rodríguez *et al.*, 2009; 2010; 2015). One interesting aspect of this plant group is the variation observed in floral shapes within syndromes. In particular, the variation in floral length (Martén-Rodríguez *et al.*, 2009; Skog, 1976) and curvature (Joly *et al.*, 2018) are two traits that have been demonstrated to be under strong genetic control (Alexandre *et al.*, 2015). Moreover, this variation parallels the variation observed in the length and curvature of the beak of hummingbirds observed across the Greater Antilles (Figure 1). In the most recent taxonomic treatment of *Gesneria* (Skog, 1976), floral evolution of the Caribbean Gesneriaceae was proposed to be influenced by their pollinators. Skog (1976) also suggested a potential correlation between the sizes and shapes of flowers and their putative hummingbird and bat pollinators, but the absence of pollinator data at that time did not allow him to formally test these hypotheses.

In this study, we used univariate measurements and geometric morphometric approaches to test if the variation in floral shape of Gesneriaceae species can be explained by the variation in the beak shape of their hummingbird pollinators. More specifically, we tested if the length, curvature and overall shape of flowers could be explained by the length, curvature and overall shape of the beak of hummingbirds. We also tested if these relationships were affected by the degree of pollination specialization of the plant species.

2.3. Methods

2.3.1. *The Antillean Gesneriaceae*

The subtribe Gesneriinae (Gesneriaceae) consists of 94 species: 65 of *Gesneria*, 25 of *Rhytidophyllum*, 2 of *Pheidonocarpa* and 2 of *Bellonia* (Clark J.L. *et al.*, 2020). All except five species occur on the Greater Antilles (Cuba, Jamaica, Puerto Rico and Hispaniola). Two species are found in the Lesser Antilles and two in northern South America. Most species can be classified into three main pollination syndromes: hummingbird pollination, bat pollination, and a mixed-pollination syndrome in which species are pollinated by hummingbirds, bats and insects (Martén-Rodríguez *et al.*, 2009). The species pollinated by hummingbirds have tubular flowers, often red or yellow (Figure 4 A, B), the species pollinated by bats have bell-shaped, green or white flowers, and the mixed-pollination species, henceforth also called generalists, have subcampanulate flowers with a constriction at the base that may be various colors, including spotted patterns (Figure 4 C). Here, we studied 18 species pollinated by hummingbirds, nine specialists and nine generalists, for which we had morphometric and pollination data (Table 1).

2.3.2. *Hummingbirds of the Greater Antilles*

At least forty species of hummingbirds (Trochilidae) can be found in the Caribbean but we only considered the hummingbird species occurring in the Greater Antilles (Table 2, information from the Global Biodiversity Information Facility (GBIF) accessed in May 2020) as no plant species from the Lesser Antilles were included in our study. Pollinator information was obtained from previous field studies (Martén-Rodríguez & Fenster, 2008; Martén-Rodríguez *et al.*, 2009; Martén-Rodríguez *et al.*, 2010; Martén-Rodríguez *et al.*, 2015). These studies showed that only seven species of hummingbirds were observed pollinating Gesneriaceae species and that in general only one or two hummingbird species pollinate any single plant species (Tables 1 and 2).

2.3.3. Corolla shape

For each plant species, corolla shape was quantified using the raw geometric morphometric data from Joly *et al.* (2018). We had photographs for 71 flowers at anthesis in longitudinal view, each with six landmarks (two at the base of the corolla, two at the tips of the petal lobes and two at the base of the corolla tube opening) and 13 semi-landmarks positioned at equal distance following the curve on each side of the corolla (Figure 5 A).

The raw landmark data was transformed by generalized Procrustes analysis in R (R core team 2014), with the geomorph R package (Adams *et al.*, 2016). The semi-landmarks were superimposed by minimizing the Procrustes distance between the reference and the target species. A principal component analysis (PCA) of the covariance matrix was performed using the ‘prcomp’ function in R and mean shapes per species were calculated from the intraspecific samples. The number of axes to keep in the following analyses was determined using the Brokenstick criteria ("vegan" package, Oksanen *et al.*, 2007).

We considered floral length and curvature as univariate characters. Flower length was measured as the distance between the base of the corolla and the aperture of the adaxial side of the corolla (further referred to as top corolla length). This measurement of flower length seems more relevant in a pollination context as it correlates more closely with the placement of the reproductive organs in this group (anthers and stigma), but we also considered the length of the abaxial side of the corolla in our analyses (bottom corolla length). Corolla lengths were measured from photographs in longitudinal view obtained from herbarium specimens (NY) or taken in the field (Gesneriaceae Image Library of The Clark Lab; <http://gesneriads.ua.edu/>), as well as from specimens of the Montreal Botanical Garden collections (Supplementary Table S1). Flower curvature was estimated from the geometric data using the angle formed between a line connecting the two landmarks at the base of the flower and another line that passes through the two landmarks at the corolla opening (Alexandre *et al.*, 2015); a greater angle indicates greater curvature (Figure 5 C).

2.3.4. *Hummingbird beak shape*

The beak shape of hummingbirds was quantified from pictures of all hummingbird species occurring on the four largest Antilles islands (e.g., Cuba, Jamaica, Puerto Rico and Hispaniola). The images of the hummingbird beaks in longitudinal view were obtained from several websites with the authorization of the photographers, and additional photographs were provided directly by photographers (Supplementary Table S2). We only retained images for which the beak of the bird was in profile view and the photograph taken perpendicularly to the beak. In total, 103 images were studied for 14 species and each species was represented by at least 6 images (Table 2). The sex of the hummingbirds was recorded for species that have a clear dimorphism. Not all species of Antillean hummingbirds studied are observed pollinators of gesneriads, but all species present in the Greater Antilles were included in the morphometric analysis to contrast the beak shapes of those species that actually visit and carry pollen from Gesneriinae flowers (pollinators) and those that were never observed visiting the study species.

The beak shape was quantified using a geometric morphometrics approach similar to the one used for the flowers and to previous studies of beak shape (Berns & Adams, 2010; Foster *et al.*, 2008). Each image was duplicated and the landmarks were positioned on each duplicate to quantify the error involved in landmark positioning. For each picture, three landmarks and 20 semi-landmarks were positioned with the software TpsDig (Rohlf, 2004). Two landmarks were placed at the base of the beak (i.e. base of the top mandible and base of the bottom mandible) and one at the tip. Ten equidistant semi-landmarks were then placed along the curve of the upper and bottom part of the beak, between the base and tip landmarks (Figure 5 B). A Procrustes analysis was done to superimpose the different beak shapes. A PCA was done on the beak landmark measurements of all the hummingbird species to illustrate the variation in beak shape among the species. The number of principal components to keep in following analyses was determined using the Brokenstick criteria as for the flower data.

Beak curvature was measured using a similar approach to the one used for corolla curvature. We measured the angle formed between lines passing through the two landmarks at the base of the beak and the two semi-landmarks adjacent to the landmark at the tip of the beak (Figure 5 D).

2.3.5. *Statistical analyses*

A Procrustes ANOVA was performed on the corolla shape data to partition the variability in corolla shape between species. Similarly, a Procrustes ANOVA was performed on beak shape data to test if the beak shape of hummingbird differs according to different factors: gender, species, and if the species is a known pollinator or not. The ANOVAs were performed using the function ‘*procD.lm*’ of the package “*geomorph*”.

To test the hypothesis that floral shape could be explained by variation in hummingbird beak shape, we considered the following three components of flower and beak shapes: length, curvature and global shape as determined by the geometric morphometric analyses using the principal components that explain a significant amount of the total variance. Only hummingbird species that are known to pollinate Gesneriads were included in beak – flower comparisons. The hypothesis was tested using different approaches depending on the shape component considered: the effect on floral length and curvature were tested using linear regressions, whereas the effect on floral shape was tested using redundancy analysis (RDA) with the ‘*rda*’ function of the “*vegan*” package (Oksanen *et al.*, 2007). The univariate and multivariate analyses were done on all species, hummingbird specialists and species with a mixed-pollination strategy. Normality of residuals was verified for all analyses.

2.4. Results

2.4.1. *Variation of the corolla shape*

The corolla morphospace illustrates variation in corolla shape among the studied species and broadly differentiates hummingbird specialist flowers from generalist flowers (Figure 6). Three principal components (which we henceforth name FL1, FL2 and FL3) were significant according to the Brokenstick criteria, which together explained 86% of the variation. The first principal

component (FL1) represents 70.8% of the total shape variance and shows variation from tubular shapes typical of hummingbird specialists to the right to subcampanulate (bell-shaped with basal constriction) corolla shapes that are characteristic of generalist flowers to the left. FL2 explains 8.4% of the variance and is characterized by the orientation of the corolla aperture and the corolla curvature. The third component explains 6.9% of the variation and represent the length and spread of the petal lobes at the corolla aperture. The Procrustes ANOVA analysis showed a strong and significant difference in shape between the two pollination strategies ($R^2=0.687$, $p<0.001$).

2.4.2. Variation in beak shape

The Brokenstick criteria suggested that the first three principal components (PC1, PC2 and PC3) explained a significant portion of the variation in beak shape and together they explain 91.5% of the total variation. The PCA shows that the different species tend to have different beak shapes (Procrustes ANOVA: $R^2=0.5787$, $p<0.001$; Figure 7). PC1 shows variation between species with curved beaks on the left (such as *Eulampis jugularis*) and species with straight beaks to the right and explains 48% of the variation. PC2 explain 27.2% of the variation and shows mainly variation in beak thickness (i.e. distance between semi-landmarks at the top and bottom of the beak). PC3 explain 16.2% of the variation and represents variation in the position of the two landmarks at the base of the beak, which is where the beak connects to the head. We did not find a significant difference in beak shape between the species pollinating Gesneriaceae and the non-pollinator species (Procrustes ANOVA: $p=0.37$).

We also tested for differences in beak shape between sexes (dimorphism) by performing a Procrustes ANOVA with the sexes nested in species and found that the shape was significantly different between sexes ($p<0.001$; Supplementary Figure S1), but this result did not hold when considering only pollinators of the Gesneriaceae ($p=0.249$).

2.4.3. *Beak-corolla correlations including all plant species*

We first tested the relationship between corolla shape and beak shape using the complete dataset, that is with both specialist and generalist plant species. When we tested the relationship between top corolla length and beak shape, we found that corolla length was significantly related to beak length ($R^2=0.211$, $p=0.031$, Table 3), and to the second principal component of the beak morphospace (PC2, $p=0.039$; Table 3). Although not significant, the regression with the overall beak shape (PC1, PC2 and PC3) explained 33% of the total variation. When using the bottom corolla length for flower length, the regression with beak length was similar but not significant ($R^2=0.177$, $p=0.051$; Supplementary Table S3). We also tested if the corolla curvature could be explained by beak shape, but none of the regressions were significant and did not explain much variation in corolla curvature (Table 3).

A multivariate redundancy analysis was also performed to test whether beak shape explains variation in floral shape as determined by the first three principal components of the corolla morphospace, but the results were not significant and the variables explained less than 10% of corolla shape variation (Supplementary Figure S2).

2.4.4. *Beak-corolla correlations for hummingbird specialists*

When only hummingbird specialists were considered, beak length, beak angle and morphometric data from landmark data explained 26%, 28% and 68% of the variation in corolla length, respectively (Table 4), although these results were statistically significant only for PC2 and PC3 on corolla length ($p=0.024$ and 0.013 , respectively; Table 4). The same tendency was found when testing which variable could explain the corolla bottom length, with each variable explaining a certain amount of the variation (21% for the beak length to 60% for the principal components) but these results are significant only for PC3 ($p=0.025$, Supplementary Table S4). The slope of the regression of the top corolla length on beak length was close to 1 (0.963, Figure 8).

Flower curvature was not significantly correlated with any variable of beak shape, although beak length explained 23% of the variation of flower curvature and the three principal components of beak shapes explained 51% of the variation (Table 4). Multivariate redundancy analyses did not show a strong relationship between corolla shape and beak length, but the variation in beak curvature and overall beak shape (morphometrics) explained 17% and 30% of the variation in corolla shape, although these were not statistically significant (Table 4; Supplementary Figure S3).

2.4.5. *Beak-corolla correlations for generalist plant species*

None of the variables tested to explain the flower shape for species with mixed-pollination systems was significant (Table 5). The slope of the regression of beak length on corolla length was close to zero, which contrasts with the values close to 1 obtained for the hummingbird specialists. The results of multivariate redundancy analysis did not show significant relationship between overall corolla shape and beak shape for generalists (Supplementary Table 5 and Figure S4). However, the length, curvature and beak morphometrics explained 11%, 14%, and 33% of the total corolla shape variation, respectively (Table 5).

2.5. Discussion

Several studies have investigated the association between beak shape and floral shape, especially looking at organ length in plant species with specialized pollination systems (Agosta & Janzen, 2005; Dohzono *et al.*, 2011; Lunau, 2004; Maglianesi *et al.*, 2014; Temeles *et al.*, 2009; Van der Niet *et al.*, 2014). Here, we investigated the relationship between the shapes of hummingbird beaks and flowers using length and curvature measurements, but also more broadly using geometric morphometrics, to compare species with specialized hummingbird pollination and species with mixed-pollination systems.

As in previous studies, we observed a positive relationship between corolla length and the length of hummingbird bills for species with specialized pollination systems with 26% of the variance

explained. However, the test was only significant when all species were considered. The lack of a significant relationship for plant specialists is possibly due to the modest sample sizes obtained by using only species with known pollinators. The slope of the regression model for beak length suggests that an increase of 1 cm in beak length is paralleled by an increase of almost 1 cm in corolla length. However, several factors could explain why the data do not fall perfectly along that model. One of these is that most hummingbirds pollinate plants with both long and short corollas (Figure 8). For example, *Anthracothorax dominicus* with a beak of 24 mm pollinates flowers with corollas that range from 19 mm (*Rhytidophyllum leucomallon*) to 29 mm (*Gesneria pedicellaris*). Departure from a perfect fit in terms of length has also been observed in several previous studies (Araujo & Sazima, 2003; Maglianesi *et al.*, 2014; Snow & Snow, 1980). But the fact that hummingbirds with long beaks have the possibility to pollinate flowers with longer corollas (Figure 8) does support a positive relationship between beak length and corolla length. Another source of variation is related to the capacity of hummingbirds to extend their tongues to reach deeper in corollas in order to reach nectar (Stiles, 1975; Temeles, 1996). Indeed, according to our results, hummingbirds tend to visit Gesneriaceae flowers with corollas that are longer than their beaks (Figure 8).

In contrast, the regression slope was close to zero for corolla and beak lengths when only species with mixed-pollination systems were considered. This suggests that the corollas of the generalist species of subtribe Gesneriinae are not under the same selection pressures from hummingbirds as their specialist relatives (Figure 8). Such results could be due to phenotypic trade-offs imposed by bats and insects that also pollinate these plants. For instance, an upper limit to corolla length, associated with overall flower size and shape, may be necessary in order to maintain successful pollination by the small *Monophyllus* bats that visit these flowers. Although this remains an hypothesis, pollinator-mediated selection is likely to explain the observed patterns, as the generalists included in this study come from groups that evolved independently from hummingbird-pollinated ancestors (Joly *et al.*, 2018; Martén-Rodríguez *et al.*, 2010). The lack of association between corolla shape and hummingbird beak for pollination generalists may not appear as a surprise, but it has been rarely documented.

We found a positive and nearly significant relationship between corolla length and beak angle for hummingbird specialists that explained 28% of the variation, suggesting that hummingbirds with

curved beaks tend to visit flowers with longer corollas. However, this relationship is confounded with that of beak length given the strong correlation between beak length and beak curvature ($r=0.742$): birds with long beaks tend to have a greater curve in their beaks. Consequently, this relationship might be a correlative result caused by the beak length – corolla length result.

Previous studies that tested the relationship between the shape of corollas with those of the hummingbird beaks have mostly used length measurements, although some have also investigated the angle of beak curvature and volume of beak and corolla (Maglianesi *et al.*, 2014; Temeles *et al.*, 2009). We decided to test whether a morphometric approach could potentially provide a more informative way to compare the shapes of flowers and pollinator beaks. The first three principal components of beak shape explained a relatively important fraction of the variation in corolla length and curvature, but mostly for the analysis of generalists and specialists taken separately. Although these results were not statistically significant, the large amount of variance explained, 30% for specialists and 33% for generalists, suggest that beak shape is related to corolla shape. These results indicate that morphometric approaches could be useful for comparing the shape of flowers and that of their pollinators.

Although beak shape dimorphism was detected amongst all Antillean hummingbirds, it was not found to be important for the pollinators of the Gesneriaceae. Strong sexual dimorphism has been shown to lead to the specialisation of plant species for a specific sex of a hummingbird species in other groups such as *Heliconia* species from the Lesser Antilles (Temeles *et al.*, 2009; Temeles *et al.*, 2010). But such processes do not seem to be important in the present group given the absence of the strong beak shape sex dimorphism as well as the lack of hummingbird sex preferences for specific plant species in the genera *Gesneria* and *Rhytidophyllum* (S. Martén-Rodríguez, pers. obs.).

In brief, the strongest association we found was that between corolla length and beak length, but only for hummingbird specialist plants and not for pollination generalists. Because of the inherent general preference of hummingbirds for small and wide flowers (Maglianesi *et al.*, 2015; Temeles *et al.*, 2009), the correspondence observed here and in previous studies between corolla and beak length is often thought to result from competition between hummingbirds for resources in nature. Clearly, the presence of other hummingbird pollinated plants and of other hummingbirds at the

same location as the studied species is expected to contribute to niche partitioning and to the general fit in size between hummingbirds' beaks and the specialist flowers they pollinate. Studies that analyze selection exerted by the different floral visitors in plant species with mixed-pollination syndromes are warranted to explore potential tradeoffs or constraints in floral evolution of Caribbean Gesneriaceae.

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2.7. Acknowledgments

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Table 1. Species of Gesneriaceae studied, their pollination mode, mean floral length (adaxial side of the corolla) and standard deviation with number of replicates for length measurements, angle of curvature with standard deviation, confirmed hummingbird pollinator(s), and island of occurrence. Abbreviations for the islands: Puerto Rico: PR, Cuba: C, Jamaica: J, Hispaniola: H and the Lesser Antilles: LA.

Species	Pollination mode	Mean Top Length (mm)	Sample size (for length)	Curvature angle	Hummingbird Pollinator	Island of occurrence
<i>Gesneria acaulis</i>	Hummingbird	44 ± 0.52	2	53.82 ± 5.92	<i>Anthracothorax mango</i>	J
<i>Gesneria citrina</i>	Hummingbird	18.7 ± 3.73	3	32.85 ± 11.13	<i>Anthracothorax viridis</i> , <i>Chlorostilbon maugaeus</i>	PR
<i>Gesneria cubensis</i>	Hummingbird	24.1 ± 5.78	5	-	<i>Anthracothorax dominicus</i>	C, H
<i>Gesneria cuneifolia</i>	Hummingbird	21.1 ± 1.32	6	20.29 ± 12.95	<i>Chlorostilbon maugaeus</i>	PR
<i>Gesneria pedicellaris</i>	Hummingbird	28.9 ± 0.18	2	37.48 ± 22.93	<i>Anthracothorax dominicus</i> , <i>Chlorostilbon swainsonii</i>	H
<i>Gesneria pulverulenta</i>	Hummingbird	14.5 ± 0.42	3	26.45 ± 1.21	<i>Chlorostilbon swainsonii</i>	H
<i>Gesneria purpurascens</i>	Hummingbird	34.1 ± 6.27	3	31.68 ± 19.76	<i>Chlorostilbon ricordii</i>	C
<i>Gesneria quisqueyana</i>	Mixed-pollination	19.9	1	39.12 ± 1.27	<i>Chlorostilbon swainsonii</i>	H
<i>Gesneria reticulata</i>	Hummingbird	18.9 ± 2.42	3	23.78 ± 10.80	<i>Chlorostilbon maugaeus</i>	PR, C, H
<i>Gesneria sintenisii</i>	Mixed-pollination	22	1	95.66 ± 2.87	<i>Chlorostilbon maugaeus</i>	PR
<i>Gesneria viridiflora</i>	Mixed-pollination	19 ± 2.94	10	68.05 ± 12.39	<i>Chlorostilbon ricordii</i>	C
<i>Rhytidophyllum auriculatum</i>	Mixed-pollination	17.7 ± 1.38	7	54.79 ± 8.04	<i>Chlorostilbon maugaeus</i>	PR, H
<i>Rhytidophyllum berterioanum</i>	Hummingbird	15.4 ± 1.43	4	51.36 ± 13.13	<i>Chlorostilbon swainsonii</i>	H
<i>Rhytidophyllum exsertum</i>	Mixed-pollination	18.7 ± 1.66	14	43.94 ± 13.47	<i>Chlorostilbon ricordii</i>	C

<i>Rhytidophyllum grandiflorum</i>	Mixed-pollination	20.9	1	53.22 ± 5.23	<i>Chlorostilbon swainsonii</i>	H
<i>Rhytidophyllum leucomallon</i>	Mixed-pollination	19.1 ± 2.83	4	41.30 ± 13.95	<i>Anthracothonax dominicus</i> , <i>Chlorostilbon swainsonii</i>	H
<i>Rhytidophyllum minus</i>	Mixed-pollination	14.8 ± 2.40	4	18.32 ± 1.78	<i>Chlorostilbon ricordii</i>	C
<i>Rhytidophyllum vernicosum</i>	Mixed-pollination	17.6 ± 3.68	2	75.95 ± 2.22	<i>Chlorostilbon swainsonii</i>	H

Table 2. Data for the hummingbirds species studied (weight, beak length, beak curvature with standard deviation, presence and type of sexual dimorphism, islands of occurrence, if it is a confirmed pollinator of Gesneriaceae, and number of photographs studied). Abbreviations for the islands: Puerto Rico: PR, Cuba: C, Jamaica: J, Hispaniola: H and the Lesser Antilles: LA. Beak size and weights of all the species come from Brown & Bowers (1985).

Species (Common name)	Scientific name	Weight (g)	Beak length (mm)	Curvature angle	Dimorphism	Island of occurrence	Pollinator confirmed	Number of photographs
Ruby throated hummingbird	<i>Archilochus colubris</i>	3.19	17.96	14.13 ± 6.21	Plumage	PR, H, C	no	7
Bee hummingbird	<i>Mellisuga helenae</i>	2.60	10.76	23.76 ± 10.08	Plumage and size	C	no	6
Green mango	<i>Anthracothorax viridis</i>	6.60 ± 0.4	24.40 ± 1.17	23.10 ± 11.39	Beak, plumage and size	PR	yes	6
Jamaican mango	<i>Anthracothorax mango</i>	7.81 ± 0.67	26.03	26.24 ± 14.09	Beak, plumage and size	J	yes	10
Purple throated carib	<i>Eulampis jugularis</i>	8.67 ± 0.56	23.59	51.17 ± 20.14	Beak, plumage and size	LA, PR	no	6
Puerto rico emerald	<i>Chlorostilbon maugaeus</i>	2.93 ± 0.2	13.62 ± 0.6	11.82 ± 8.67	Beak, plumage and size	PR	yes	8
Hispaniolan emerald	<i>Chlorostilbon swainsonii</i>	4.85	17.30	12.32 ± 12.34	Beak, plumage and size	H	yes	6
Red billed streamertail	<i>Trochilus polytmus</i>	4.10	19.80	32.86 ± 15.51	Beak, plumage and size	J	yes	10
Green throated carib	<i>Eulampis holosericeus</i>	5.60 ± 0.40	22.74 ± 2.20	46.09 ± 9.21	Beak, plumage and size	LA,PR	no	8
Antillean crested hummingbird	<i>Orthorhyncus cristatus</i>	2.71 ± 0.18	10.72 ± 1.18	14.43 ± 8.53	Plumage and size	LA,PR	no	7
Cuban emerald	<i>Chlorostilbon ricordii</i>	4.23	17.20	26.03 ± 12.68	Beak, plumage and size	C	no	9
Vervain hummingbird	<i>Mellisuga minima</i>	2.43 ± 0.10	10.45	20.04 ± 11.50	Size	H,J	yes	7
Antillean mango	<i>Anthracothorax dominicus</i>	5.66 ± 0.41	24.13 ± 0.75	37.49 ± 14.09	Beak, plumage and size	H, PR	yes	7

Table 3. Relationship between the beak shape of hummingbirds and the corolla shape of plant species with both specialist and generalist pollination strategies as determined by regression (simple, multiple regressions and redundancy analysis).

	Variable(s) tested	Estimate	P-value	R ²
Corolla length (top)	beak length	0.812	0.031 *	0.211
	beak angle	0.248	0.118	0.117
	PC1+PC2+PC3		[p-value modèle]	0.334
	PC1 PC2 PC3	-20.311 596.182 -241.602	0.792 0.039 * 0.175	
Corolla curvature	beak length	-0.268	0.822	0.003
	beak angle	-0.263	0.608	0.015
	PC1+PC2+PC3			0.031
	PC1 PC2 PC3	158.911 462.668 -92.455	0.628 0.631 0.887	
FL1+FL2+FL3	Length		0.404	0.040
	Curvature		0.604	0.024
	PC1+PC2+PC3		0.951	0.039

Table 4. Relationship between the beak shape of hummingbirds and the corolla shape of plant species with a specialist pollination strategy as determined by regression (simple, multiple regressions and redundancy analysis).

	Variable(s) tested	Estimate	P-value	R ²
Corolla length (top)	beak length	0.963	0.075	0.260
	beak angle	0.445	0.0638	0.278
	PC1+PC2+PC3			0.676
	PC1 PC2 PC3	47.467 735.338 -741.998	0.605 0.024 * 0.013 *	
Corolla curvature	beak length	1.251	0.136	0.229
	beak angle	0.227	0.615	0.029
	PC1+PC2+PC3			0.515
	PC1 PC2 PC3	19.091 1042.228 -16.214	0.930 0.088 0.974	
FL1+FL2+FL3	Length		0.487	0.078
	Curvature		0.157	0.176
	PC1+PC2+PC3		0.362	0.301

Table 5. Relationship between the beak shape of hummingbirds and the corolla shape of plant species with a generalist pollination strategy as determined by regression (simple, multiple regressions and redundancy analysis).

	Variable(s) tested	Estimate	P-value	R ²
Corolla length (top)	beak length	-0.071	0.776	0.011
	beak angle	-0.068	0.369	0.102
	PC1+PC2+PC3			0.249
	PC1	-9.597	0.837	
	PC2	-114.159	0.614	
	PC3	101.565	0.340	
Corolla curvature	beak length	-3.188	0.232	0.173
	beak angle	-0.921	0.267	0.151
	PC1+PC2+PC3			0.309
	PC1	123.748	0.819	
	PC2	-2246.824	0.361	
	PC3	949.193	0.424	
FL1+FL2+FL3	Length		0.106	0.106
	Curvature		0.142	0.141
	PC1+PC2+PC3		0.328	0.327

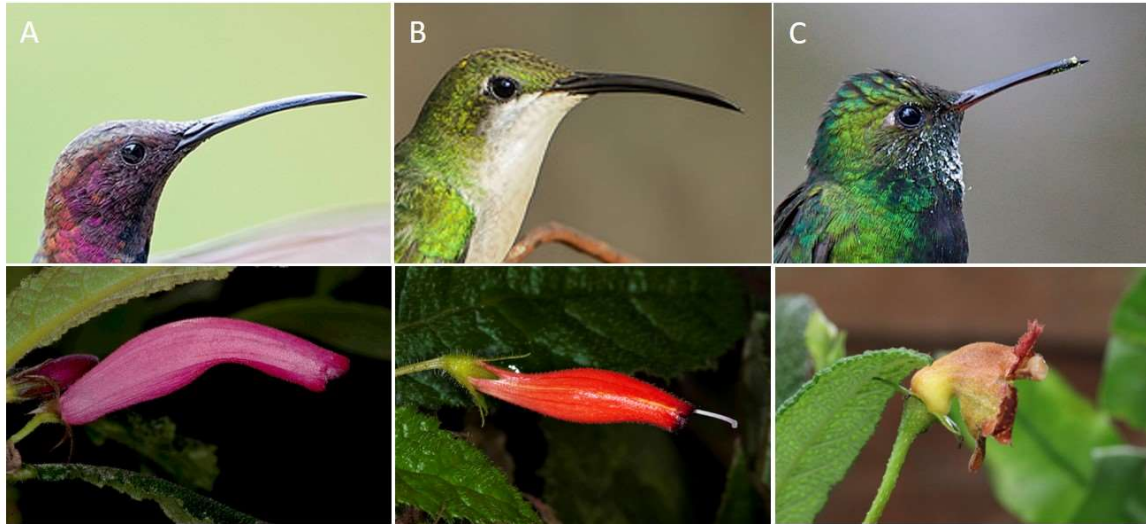


Figure 4. Profile pictures of the beak of different species of hummingbirds of the Carribean showing the variation of beak shape between species, and profile view of the corolla of the Gesneriaceae they pollinate. (A) *Anthracothorax mango* pollinates *Gesneria acaulis*, (B) *Anthracothorax dominicus* pollinates *Gesneria pedicellaris* and (C) *Chlorostilbon swainsonii* pollinates *Rhytidophyllum vernicosum* Photo credits: Hummingbirds: Rafy Rodriguez, Gesneriaceae: John J Clark, Simon Joly

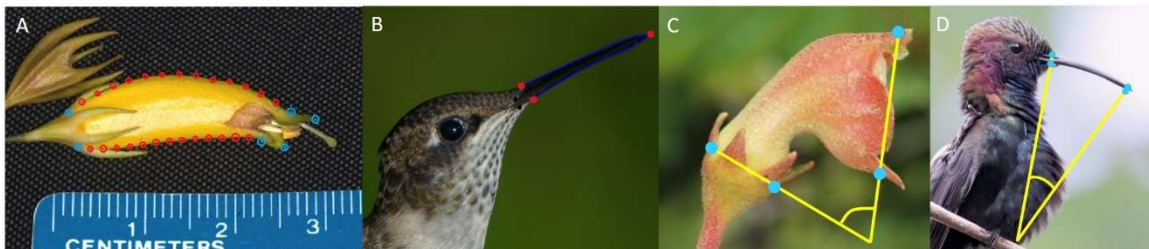


Figure 5. A) Example of landmark (blue dots) and semi-landmarks (red dots) positioning for the geometric morphometric analysis of floral shape ; B) Example of landmark (red dots) and curve for semi-landmarks (blue line) positioning for the geometric morphometric analysis of beak shape ; C) Example of measurement of corolla curvature with landmarks (base and opening of the corolla) of the geometric morphometric data (blue dots) ; D) Example of the measurement of beak curvature with landmarks (base of the beak) and semi-landmarks (next to the tip of the beak) (blue dots).

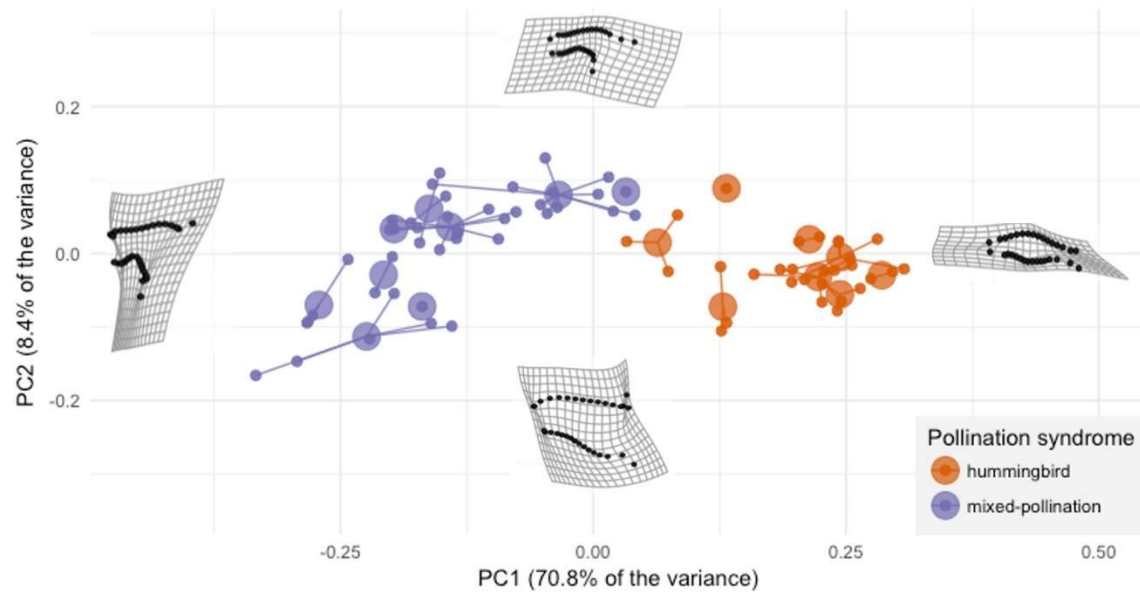


Figure 6. Principal Component Analysis of floral traits that determine corolla shape. Large dots represent the means for each species and they are connected by lines to small dots that represent the floral shapes of the individuals belonging to each species. Thin-plate spline deformation grids show corolla shape variation among the principal components, plus or minus 2 standard deviation from the mean shape.

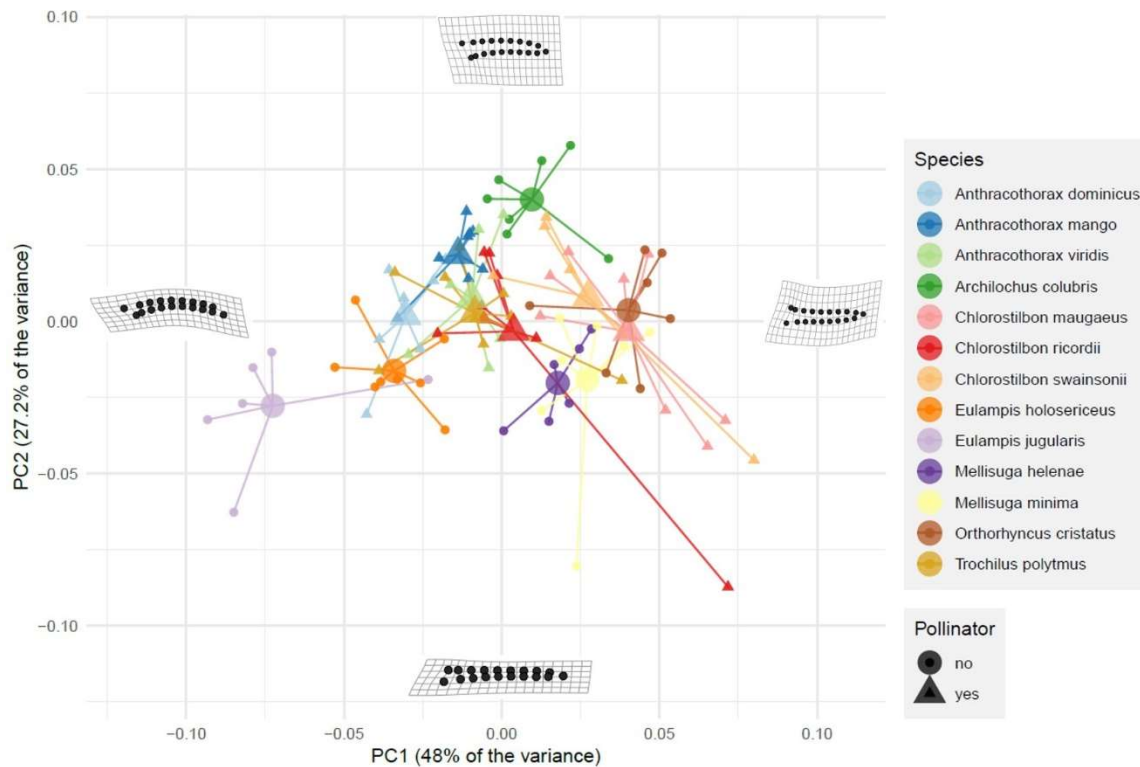


Figure 7. Principal Component Analysis of beak shape traits of hummingbirds from the Greater Antilles, with confirmed pollinators highlighted by a triangle shape. Species are differentiated by colors. Large dots represent the means for each species, which are connected by lines to small dots that represent the beak shapes of the individuals belonging to each species. Thin-plate spline deformation grids show beak shape variation among the principal components, plus or minus 2 standard deviation from the mean shape.

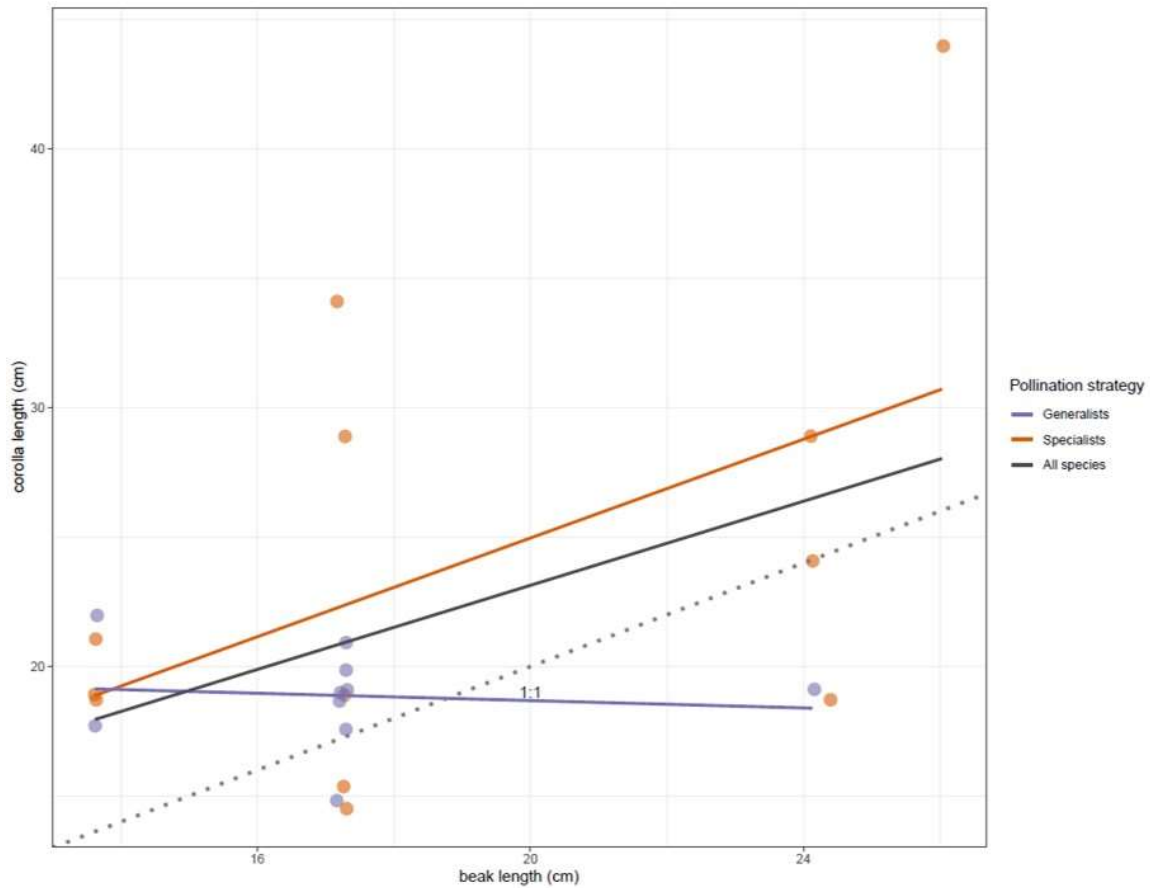


Figure 8. Plot showing the relationship between the top corolla length and the beak length for all plant species (generalist and specialist) (black line), hummingbird specialist plant species (orange line), and only generalist plant species (purple line). The black dot line corresponds to the 1:1 line. Random noise was added to the points (jitter) to facilitate visualization.

Chapitre 3 : Efficacité de pollinisation d'une espèce généraliste en Haïti

Titre original: Pollinator performance of the pollination generalist *Rhytidophyllum bicolor* (Gesneriaceae) in Haiti 15 months after Hurricane Matthew

Statut : Sous presse, Selbyana

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Contribution des auteurs :

Julie Faure et Simon Joly ont conçu l'étude, collecté les données et analysé les données. Julie Faure a écrit la forme initiale du manuscrit et tous les auteurs ont contribué à la version finale.

3.1. Abstract

Pollination performance consists of the visitation rate and efficiency of animal species pollinating a given plant species, and it is central to understanding the contribution of pollinators to the evolution of species. We studied the pollination performance of different floral visitors of *Rhytidophyllum bicolor* Urb. (Gesneriaceae), a species endemic to southwest Haiti for which no prior pollination information existed. Although pollinator visitation rates are known for several Antillean Gesneriaceae, single visit efficiency has never been estimated and pollination performance is unknown in the group. We found that bats were more frequent and more effective pollinators than bees, and thus had a greater pollination performance even if the contribution of bees is not negligible. Hummingbird performance could not be estimated because no pollination was observed in this study although they had been observed in previous field trips. This is likely because hummingbird populations may have been strongly impacted by Hurricane Matthew that hit the region in October 2016, 15 months prior to this study. These results highlight the advantages of being a pollination generalist to ensure good reproductive success even in the absence of a pollinator, a strategy potentially important in the Caribbean islands that are frequently affected by natural disasters such as hurricanes.

3.2. Introduction

Without the assistance of animals to ensure their pollination, roughly 88% of angiosperms would not be able to complete their reproductive cycle (Ollerton *et al.*, 2011). This mutualism sometimes leads to the specialization of plant species to a few pollinator species, or to generalization where plants are effectively pollinated by several pollinators (Waser, 2006; Waser *et al.*, 1996). Such ecological strategies have a strong impact on floral evolution, which is expected to be affected by the composition of pollinator guilds and, more specifically, by the relative performance of each pollinator (Aigner, 2001, 2006). For instance, floral traits that favour one pollinator might be detrimental to another one, such as narrow corolla tubes that are thought to increase hummingbird pollination and reduce bee pollination (Castellanos *et al.*, 2004), although empirical studies have yet to find strong support for such fitness trade-offs (Aigner, 2004; Castellanos *et al.*, 2004; Muchhala, 2007b; Sahli & Conner, 2011).

In order to better understand the reproduction of plant species and the relative importance of their different pollinators, it is important to estimate their performance (Freitas, 2013; Ne'eman *et al.*, 2010). Pollinator performance (also called pollinator importance) consists of two main components: 1) the visitation rate or the frequency at which a pollinator makes contact with the flower reproductive organs, and 2) the pollinator efficiency, which is the capacity of a pollinator to remove pollen from the anthers (male component), transfer it to the stigma, and produce seeds (female component) following a single pollinator visit (Armbruster, 2014; Freitas, 2013; Ne'eman *et al.*, 2010). Note that the female reproductive success (seed set produced) is sometimes estimated from the number of fertilized ovules or the number of pollen grains deposited on the stigma (Ne'eman *et al.*, 2010). Ideally, pollinator performance for a plant should be estimated over its whole life, but this is more difficult to evaluate for perennial plants.

Many pollination studies limit their observations to visitation rates only. While this provides information about the pollinator guild of the plant, this information might not reflect the actual contribution of each pollinator towards the reproductive success of the plant. For this reason, it is important to measure pollinator performance because many studies have shown that, contrary to what was proposed by Stebbins (1970), the most frequent pollinator is not always the most effective

(Fumero-Cabán & Meléndez-Ackerman, 2007; Mayfield *et al.*, 2001; Niemirski & Zych, 2011; Sahli & Conner, 2007; Zych, 2007).

Pollination generalism, for which pollinator performance is particularly relevant because several species contribute to the reproduction of the plant, represents a common pollination strategy in angiosperms (Ollerton *et al.*, 2007; Waser *et al.*, 1996). There is no universal definition of a pollination generalist, but many authors agree that they describe plant species pollinated by two or more distinct functional pollinators (Gómez & Zamora, 2006; Johnson & Steiner, 2000; Ollerton *et al.*, 2007). If pollination specialists have been widely studied (Armbruster *et al.*, 2000; Herrera, 1996; Johnson & Steiner, 2000; Thompson, 1994), generalists have received considerably less attention despite their abundance (but see Aigner, 2004; Gómez *et al.*, 2014; Sahli & Conner, 2007).

In this study, we investigate the pollinator performance of *Rhytidophyllum bicolor* Urb. (Gesneriaceae), a species endemic to Haiti for which no pollination data exist. Considering its subcampanulate corolla (bell shape with a constriction at the base of the corolla; Figure 9 C), we hypothesize that *R. bicolor* is probably a pollination generalist as corolla shape is a very good predictor of pollination strategy in the group (Joly *et al.*, 2018; Martén-Rodríguez *et al.*, 2009). The subtribe Gesneriinae, to which *R. bicolor* belongs, has indeed been the subject of several pollination studies (Martén-Rodríguez & Fenster, 2008; Martén-Rodríguez *et al.*, 2009; Martén-Rodríguez *et al.*, 2010; Martén-Rodríguez *et al.*, 2015), although none have estimated pollinator efficiency. These have shown that pollination generalists in the Gesneriinae are normally pollinated by hummingbirds, bats and insects, three functionally distinct pollinators. Interestingly, pollination generalists in the New World Gesneriaceae appear to be restricted to the Antilles (Martén-Rodríguez *et al.*, 2015) and have been shown to have evolved several times independently (Joly *et al.*, 2018; Martén-Rodríguez *et al.*, 2010). Generalist pollination strategies could be particularly effective on islands by providing reproductive insurance and by reducing the likelihood of local extinctions (McKinney, 1997; Raia *et al.*, 2016). This is because islands generally have lower pollinator richness compared to nearby continents (Barrett *et al.*, 1996; Olesen *et al.*, 2002) and have pollinator communities that vary through time due to migrations and natural disasters such as hurricanes (Armbruster & Baldwin, 1998; Martén-Rodríguez *et al.*, 2010). In this particular study, our investigation of pollination performance on *R. bicolor* occurred 15 months after Hurricane Matthew in October 2016.

3.3. Materials

3.3.1. Studied species

***Rhytidophyllum bicolor*.** *Rhytidophyllum bicolor* is endemic to the Massif de la Hotte in South-West Haiti on the Tiburon peninsula (Figure 9 A), where it is locally abundant. It is a shrub up to 2 m tall that produces several cymose inflorescences (Figure 9 B) throughout the year. Flowers are protogynous with temporally separated female and male stages that last one day each. The dehiscent capsules release a few hundred seeds that drop directly onto the ground.

Pollinators. The pollinators of *Rhytidophyllum bicolor* were not known before this study, although hummingbirds were observed (S. Joly, pers. obs.). The following four species of hummingbirds present at the study site are possible pollinators: *Chlorostilbon swainsonii* (Lesson, 1829) (Trochilidae) (Peguero *et al.*, 2006), *Mellisuga minima* (Linnaeus, 1758) (Trochilidae), *Archilochus colubris* (Linnaeus, 1758) (Trochilidae) and *Anthracothorax dominicus* (Linnaeus, 1766) (Trochilidae) (Figure 10 A, B, C, D). Two species of nectarivorous bats occur at the study site and are potential pollinators: *Monophyllus redmani* Leach, 1821 (Phyllostomidae) and *Phyllonycteris poeyi* Gundlach, 1861 (Phyllostomidae) (Figure 10 E, F). Finally, bees are known to be occasional pollinators of generalist species of Antillean Gesneriaceae (Martén-Rodríguez *et al.*, 2015) and they could thus also pollinate *R. bicolor*.

3.3.2. Research site

The study was conducted in the Pic Macaya National Park in southern Haiti from January 19th to January 28th 2018. The park area is more than 8,000 hectares, where unexploited cloud forest can still be found. Elevation in the park reaches a maximum altitude of 2,347 meters above sea level at Pic Macaya. This is the most important protected area of the country and one of the largest centers of endemism in Haiti (Peguero *et al.*, 2006). Observations were carried out around the village of Formon (latitude 18.324249, longitude -74.009565) and in Bois Formon, Bois Cavalier and Fonblé, at elevations between 900 and 1150 meters in humid karst forest and in disturbed areas

where *R. bicolor* is abundant. This “buffer zone” of the park is highly disturbed and strongly affected by deforestation (Hedges *et al.*, 2018) and farming (pers. obs.).

3.4. Methods

3.4.1. Pollination observations

Each observation period lasted from 1 to 4 hours and was done 2-3 meters from the plant. Every studied population had several flowers at anthesis. Daily observations were done between 6 am and 4 pm on different populations. Night observations were performed from 6:30 pm to 11 pm using red light flashlights. A camera with night vision (Sony HDR-CX550V) also was used some nights to record the contact of bats with the reproductive parts (Cárdenas *et al.*, 2017; Muchhala & Potts, 2007). At the beginning of each observation period, we noted the total number of flowers available, the number of flowers in female phase and thus with receptive stigma, and we removed the stigmas of flowers that had already received pollen (determined visually).

3.4.2. Pollinator performance

We followed Freitas (2013) and estimated the pollinator performance (called pollinator effectiveness by Freitas) as the visitation rate multiplied by the single visit efficiency (i.e. pollinator efficiency). Visitation rate is the number of times a pollinator contacts the reproductive organs of one specific flower per hour on average. As the visitation rate often conforms to a Poisson distribution, a 95% confidence interval was calculated with the exact method because of our small sample sizes (Ulm, 1990). The pollinator efficiency was estimated by the mean number of pollen grains deposited on the stigma after a single pollinator visit (Olsen, 1996; Park *et al.*, 2016; Rogers *et al.*, 2013; Thomson & Goodell, 2001).

To count the number of pollen grains deposited on the stigma after a single pollinator visit, stigmas were removed from the flower immediately following the visit and placed in a tube containing 70% isopropyl alcohol and brought back to the laboratory. In the laboratory, the tube was vortexed for 30 seconds to remove all the pollen from the stigma. The stigma was then removed and the tube was weighed to calculate the total volume of alcohol in the tube using the volumetric mass of 70% isopropyl alcohol. The number of pollen grains in 2 μ L of solution was counted using a haemocytometer from ten replicates per tube of alcohol, with five replicates pipetted from the top of the tube and five from the bottom immediately after vortexing. The total number of pollen grains in each tube was then estimated by multiplying the mean number of pollen grains in 2 μ L by the dilution ratio. A Mann-Whitney-Wilcoxon test was performed to compare the mean pollen deposited by each pollinator.

3.5. Results

We performed 23 hours of day observations and 18 hours of night observations over ten days of field work, from January 19th to January 28th 2018. Nine populations were studied and the number of receptive flowers in each population varied between 3 and 17 (Supplementary material). Bee pollination observations came from two distinct populations while all bat pollinations were observed in a single population. Consequently, we could not properly assess the variation among populations. Bats were the most abundant pollinators with a visitation rate of 0.121 visits per hour per flower with 95% confidence interval (CI) [0.0739, 0.182] (Table 6), however, species identification could not be confirmed with the video camera. Bee pollination of *R. bicolor* was rarer, with a visitation rate of 0.0176 95% CI [0.0048, 0.045] visits per hour per flower. We were not able to photograph or capture bees, so their identification is tentative. Bees appeared to represent members of the genus *Anthophora* based on the color and shape of the head, thorax and abdomen, and their size.

No hummingbird was observed pollinating *R. bicolor*, which results in a visitation rate of 0. However, hummingbirds were virtually absent from the park. Only 4 individuals (three *Mellisuga minima* and one *Archilochus colubris*) were observed during our ten days of field work in the park. Hummingbirds were observed to be abundant in the park during a previous research expedition (S.

Joly, pers. obs., 2014). Drastic changes in the number of hummingbird populations is also supported by the testimony of four park guides hired during our research expedition.

Three types of pollen were observed on the slides. Gesneriaceae pollen is easily recognised by its small, pale, oval-shaped tricolporate pollen with three prominent colpi. Two other types of pollen were also observed but could not be identified. One was large, dark and circular (henceforth called unknown pollen 1) and the other had a triangular shape and ornamentations (unknown pollen 2). The pollen of different Gesneriaceae species is difficult to distinguish under a microscope (Beaufort-Murphy, 1983), but other species were relatively rare in the park and were not present within 500 m of the studied plants.

Of the three types of pollen observed, the Gesneriaceae pollen was the most abundant (Figure 11). The maximum number of pollen grain deposited for the unknown pollen 1 and 2 was 9.2 (standard error (se) = 2.05) and 35 (se = 35.19) respectively, but they were often completely absent on sampled stigmas. Pollen of *R. bicolor* was deposited by both bats and bees, while the two other types of pollen were only deposited by bats (Figure 3). The Gesneriaceae pollen laid on the stigma by bats per visit (mean = 21868, se = 4648, min = 4675, max = 44945) was more abundant than the pollen deposited by bees (mean = 4813, se = 2592 min = 1945, max = 12572; Mann-Whitney-Wilcoxon test, $p=0.04176$). For both pollinators, the amount of pollen deposited varied strongly between visits.

The pollinator performance of bats was 2646 pollen grains deposited per flower per hour on average, versus 84 pollen grains deposited per flower per hour for bees (TABLE 1). For hummingbirds, pollinator performance could not be measured as we did not observe any hummingbird visits.

3.5.1. Pollinator behaviour

Bats first started to be seen around *R. bicolor* after sunset, between 7pm and 8pm. At first, the bats were only observed flying around the plant before starting to pollinate them. Visitation sometimes

occurred repeatedly on the same flower (flowers not receptive anymore of with the stigma removed still offer nectar), sometimes within a short time lapse. The bat contacted the flower in a fraction of a second for each visit and then left without visiting another flower from the same population. In contrast, bees tended to stay a relatively long time (ca. one minute) on a flower collecting pollen, and generally passed to a neighbouring flower (traplining). We know from previous observation-based studies (S. Joly pers. obs., 2014) that hummingbirds also pass from one flower to another of the same population (not always from the same plant) when they pollinate.

3.6. Discussion

We studied the pollination biology of *Rhytidophyllum bicolor*, a species endemic to Haiti for which no pollination data was previously available. Combined with previous partial observation data, observations have shown that *R. bicolor* is pollinated by bats, hummingbirds and bees. Given that *R. bicolor* is visited and likely pollinated by functionally distinct pollinators, it can be considered a generalist. Yet, because of the small number of species involved, this strategy is sometimes called mixed-pollination or multimodal (e.g., bimodal) pollination (Gómez & Zamora, 2006; Herrera, 2005; Niemirski & Zych, 2011; Ollerton *et al.*, 2007). This mixed-pollination strategy with hummingbirds, bats and bees is frequent in Antillean Gesneriaceae (Martén-Rodríguez & Fenster, 2008; Martén-Rodríguez *et al.*, 2009; Martén-Rodríguez *et al.*, 2015) and has been shown to have evolved several times independently (Joly *et al.*, 2018; Martén-Rodríguez *et al.*, 2010).

Our study is the first in Antillean Gesneriaceae to quantify single visit efficiency of pollinators and calculate pollinator performance. This information is important to understand the role that different pollinators can play on the evolution of flowers in a species (Armbruster, 2014; Freitas, 2013; Ne'eman *et al.*, 2010). Unfortunately, we were not able to estimate single visit efficiency for hummingbirds. We did, however, find that bats were more efficient than bees for depositing pollen on the stigma of *R. bicolor*. In terms of overall pollinator performance, bats were better than bees with both a higher visitation rate and higher pollinator performance. Nectarivorous bats are important pollinators in the tropics and have been shown to be effective pollinators on generalist species (Aguilar-Rodríguez *et al.*, 2016; Nassar *et al.*, 1997), sometimes more than hummingbirds (Muchhala, 2003; Muchhala & Thomson, 2010; Queiroz *et al.*, 2016) even if they are less frequent

visitors (Law & Lean, 1999). We note, however, that single-visit efficiency of bees is not negligible for *R. bicolor*, as is the case for other pollination generalists (Aguilar-Rodríguez *et al.*, 2016; Nassar *et al.*, 1997). Indeed, even if visits of bees are rare, the pollen deposited in a single visit has the potential of fertilizing a good fraction of the thousands of ovules present in the ovary of each flower and do contribute to the reproduction of *R. bicolor*. Although the number of ovules per flower is not known for *R. bicolor*, it varies between 1700 and 3000 for two new world Gesneriaceae investigated, *Besleria trifolia* and *Drimonia rubra* (Feinsinger *et al.*, 1986). If these numbers are indicative of ovule numbers in *R. bicolor*, the number of pollen grains deposited by bees could well exceed the number of ovules per flower in most visits. Note, however, that the amount of deposited pollen represents the female function of the flower. Because bees collect important amounts of pollen grains for consumption they could have a negative impact on the male flower function and further reduce their importance as pollinators compared to hummingbirds.

Unfortunately, we were unable to estimate the single visit efficiency and the pollinator performance of hummingbirds because they did not pollinate *R. bicolor* during our study. This is clearly a consequence of the low population densities of hummingbirds at the time of the study, which were likely the result of the passage of Hurricane Matthew through the Pic Macaya Park on October 4th, 2016, with winds over 240 km/h. Based on previous pollination studies of Antillean Gesneriaceae, we would have expected hummingbirds to have visitation rates very similar to that of bats (Martén-Rodríguez & Fenster, 2008; Martén-Rodríguez *et al.*, 2009; Martén-Rodríguez *et al.*, 2015), but further studies when hummingbird populations have recovered will be needed to estimate the pollination performance of hummingbirds for *R. bicolor*.

Many studies have proposed that the higher preponderance of generalist pollination strategies on islands compared to the continent could be at least partially explained by the temporal variation of pollinator populations (Armbruster & Baldwin, 1998; Gómez & Zamora, 2006; Martén-Rodríguez *et al.*, 2009; Waser *et al.*, 1996; Wiley & Wunderle, 1993). Indeed, Caribbean islands are known to be subjected to frequent natural catastrophes (Wiley & Wunderle, 1993). Our study is a good example; Hurricane Matthew had a strong impact on hummingbirds populations, likely through direct mortality as well as indirect mortality due to depleting part of their food sources via the loss of flowers (Donihue *et al.*, 2018; Spiller *et al.*, 1998; Wiley & Wunderle, 1993; Willig *et al.*, 2010). By measuring the presence of pollinators, their efficiency and their visitation rate, the recovery of

pollinator populations could be better understood, as was done by Wiley & Wunderle (1993). Unfortunately, there was no quantification of the hummingbirds population before Hurricane Matthew, but personal observations (S. Joly, 2014) and testimonies by park rangers confirmed a huge drop in hummingbird abundance. It is in such situations where a generalist pollination strategy becomes advantageous. Indeed, even if the hummingbird populations in the park Pic Macaya were almost completely depleted by Hurricane Matthew, *R. bicolor* could still rely on its other pollinators for its reproduction. The bat populations were also strongly affected by the Hurricane when compared to their abundance in 2014 (S. Joly, unquantified pers. obs.), and as such visitation rates might be affected. But bats were still sufficiently abundant in 2018 to pollinate *R. bicolor* and consequently ensure its reproduction. Although pollination generalist strategies are frequent in Antillean Gesneriaceae, none have yet to be reported from the continent (Martén-Rodríguez *et al.*, 2015). These observations could be potentially linked to the fact that ecological generalists tend to have lower extinction rates in general (McKinney, 1997; Raia *et al.*, 2016).

Further studies are needed to measure the performance of pollinators in specialist and generalist plant species and to better understand their role in the evolution of species at both microevolutionary and macroevolutionary levels. The Antillean Gesneriaceae represents an ideal group for such studies because it allows for comparison of pollinator efficiencies among pollination strategies, between species with a given strategy, and on different islands. Additionally, as hummingbird and bat populations are likely to eventually return to pre-hurricane levels, future pollination studies of *R. bicolor* could contribute to better understand the fluctuation of hummingbird and bat populations in the Antilles and their impact on the reproductive strategy of *R. bicolor*.

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Table 6. Pollinator mean visitation rate, mean single visit efficiency and overall pollinator performance. Sample sizes are indicated for visitation rates (number of visits) and single visit efficiency (number of pollinations). The 95% confidence intervals are indicated and estimated from the expectation from a Poisson distribution for the visitation rate and from standard errors for the single visit efficiency.

Pollinator	Bats	N (bats)	Bees	N (bees)
Visitation rate <i>Mean pollinator visits per flower per hour</i>	0.121, 95% CI [0.0739, 0.182]	21 visits	0.0176, 95% CI [0.0048, 0.045]	4 visits
Single visit efficiency <i>Mean number of pollen grains deposited on the stigma per visit</i>	21868, 95% CI [12758, 30978]	12 pollinations	4813, 95% CI [0, 9893]	4 pollinations
Pollinator performance <i>Mean number of pollen grains deposited per flower per hour</i>	2646	n.a.	84.71	n.a.

A GBIF occurrences for *Rhytidophyllum bicolor*

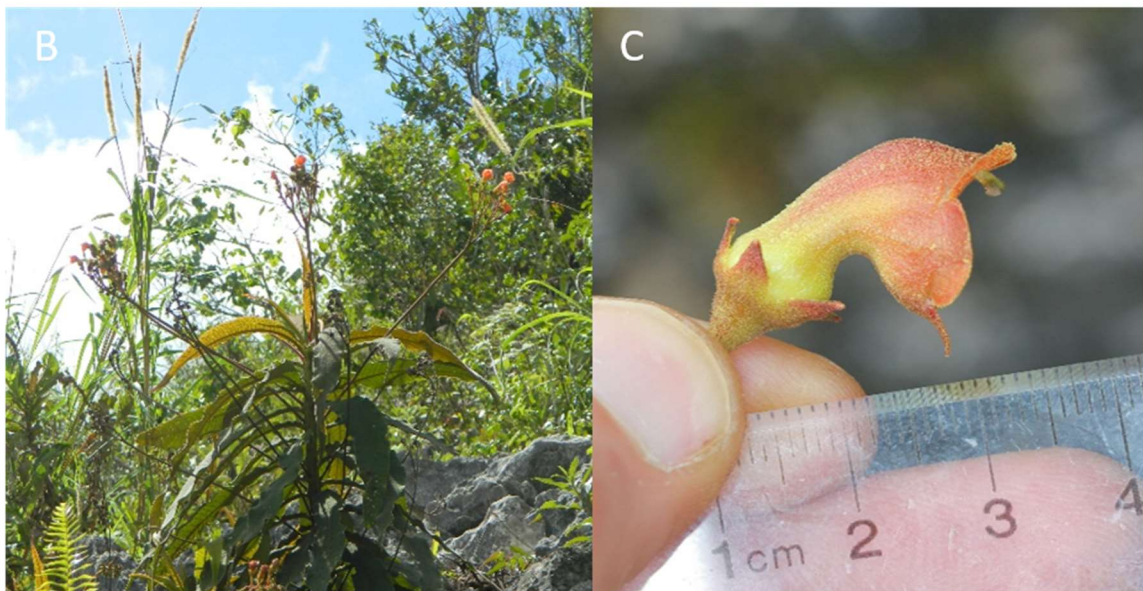
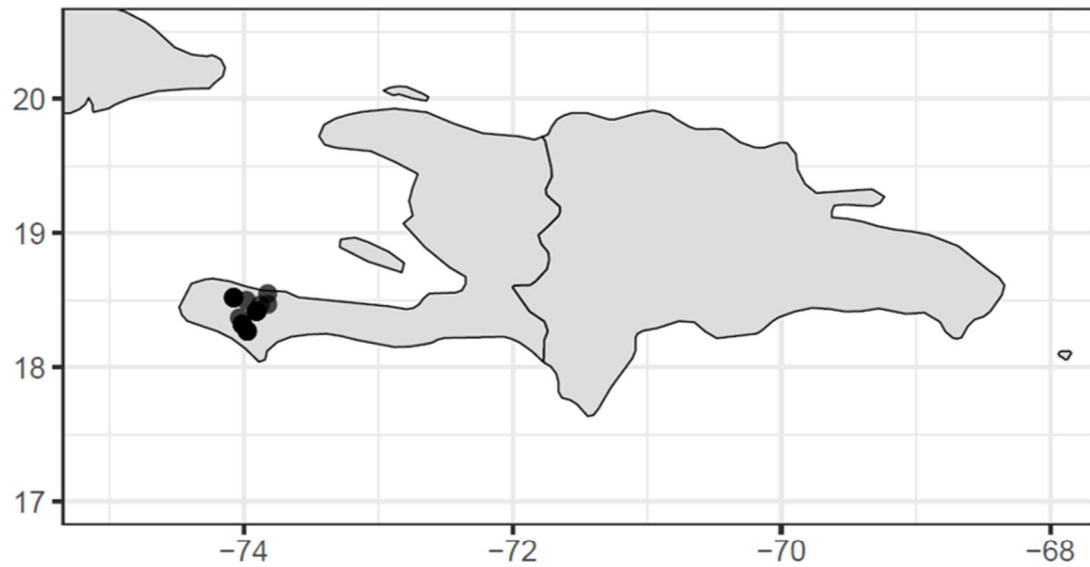


Figure 9. Pictures of a *Rhytidophyllum bicolor* distribution (A) estimated from georeferenced accessions obtained from GBIF (Global Biodiversity Information Facility; accessed March 30th, 2020), plant (B) and flower (C). Photo credits: J. Faure (B) and S. Joly (C).

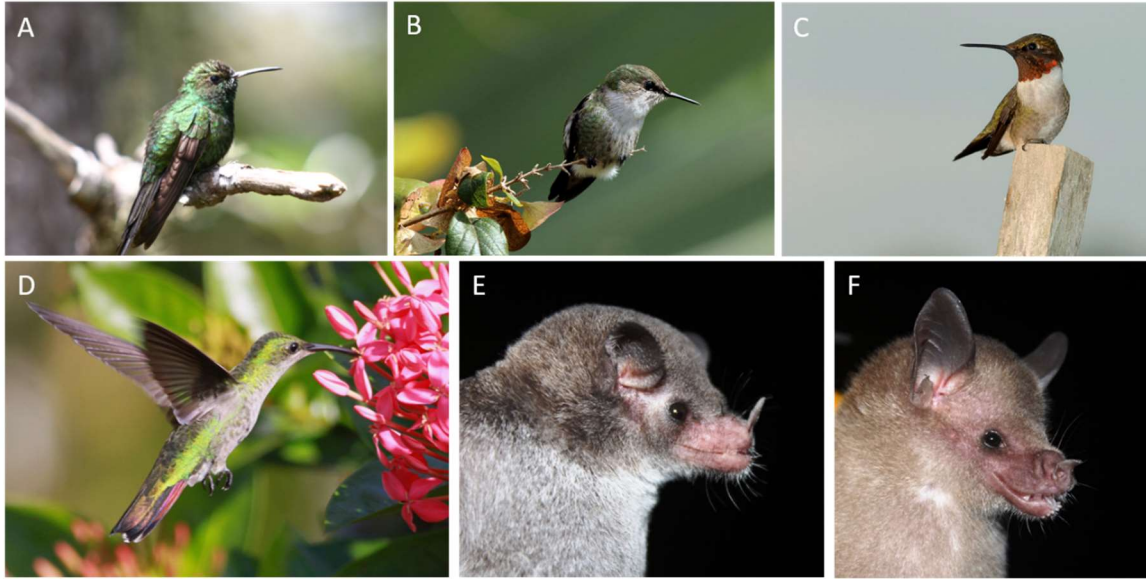


Figure 10. Pictures of putative pollinators of *R. bicolor*: A) *Chlorostilbon swainsonii* © Silvana Marten-Rodriguez, B) *Mellisuga minima* © Charles J. Sharp (CC BY-SA 4.0), C) *Archilochus colubris* © Joe Schneid (CC BY 3.0), D) *Anthracothorax dominicus* © zankaM (CC BY-SA 3.0), E) *Monophyllus redmani* © Joaquín Ugarte (CC BY-NC 4.0)(CC BY-NC 4.0) and F) *Phyllonycteris poeyi* © Joaquín Ugarte, some rights reserved (CC BY-NC).

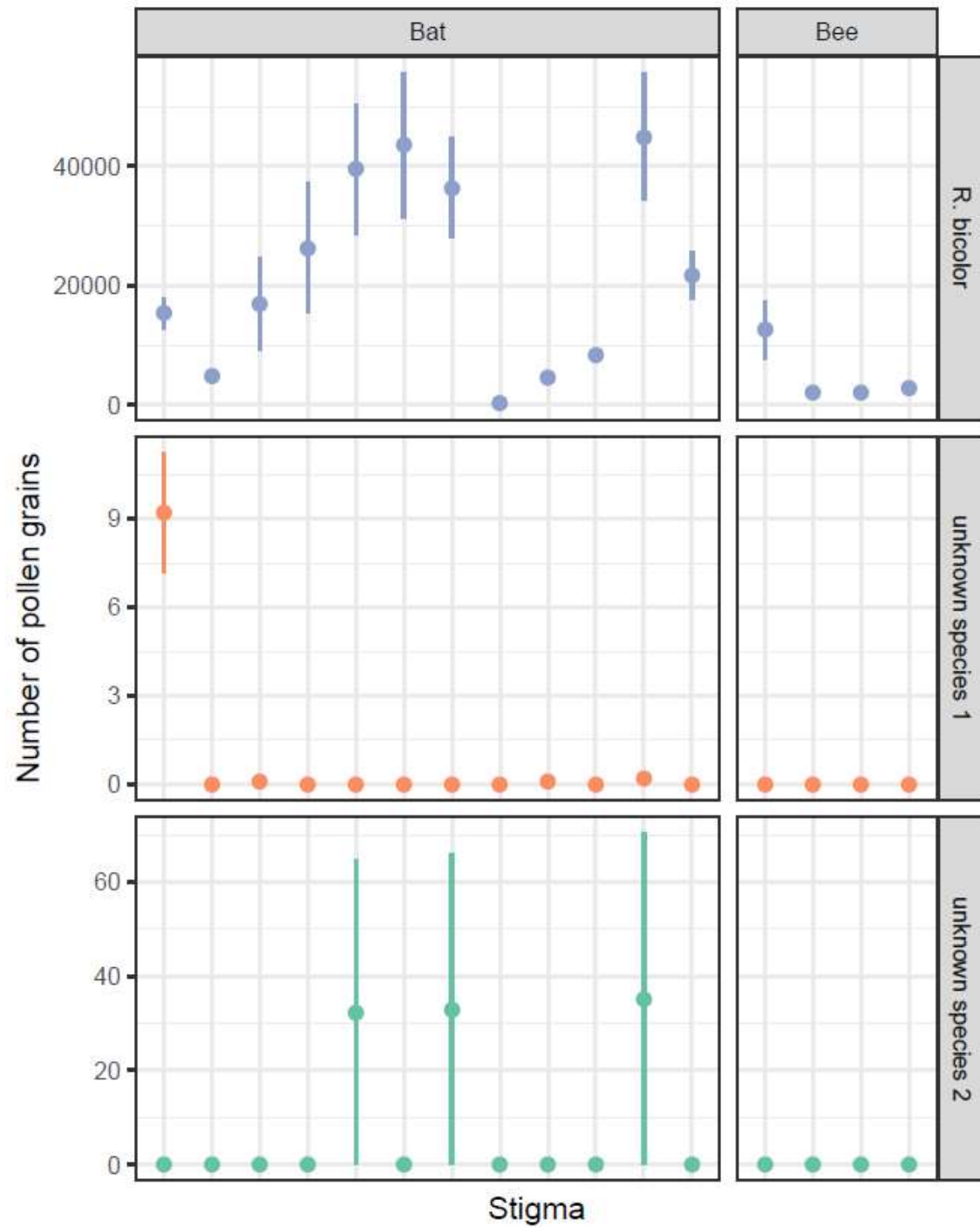


Figure 11. Estimates of pollen grains of *R. bicolor* and two unknown pollens deposited during a single visit by each type of pollinator. Each point column in the graph represents a different stigma. Errors bars represent the standard errors of the technical replicates.

Chapitre 4 : Effet de l'urbanisation sur la forme florale chez *Impatiens capensis*

Titre original : Urbanization affects the flower shape of *Impatiens capensis* through its impact on pollinator communities

Statut : En préparation

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Contribution des auteurs :

Julie Faure et Simon Joly ont conçu l'étude. Julie Faure a collecté les données. Julie Faure et Valentine Voltz ont réalisé la morphométrie. Julie Faure et Simon Joly et Valentine Voltz ont analysé les données. Julie Faure a écrit la version initiale du manuscrit et tous les auteurs ont contribué à l'écriture de la version finale.

4.1. Abstract

Floral shape is a critical trait for the reproduction of plants pollinated by animals. Its variation is strongly affected by the biotic environment of species (pollination, herbivory), but also potentially by their abiotic environment (climate, soil, urbanization). In the last two decades, numerous studies have assessed the impact of urbanization on pollinator communities as well as on many plant reproductive traits, but few have investigated its impact on floral shape. In this study, we tested the effect of urbanization on the floral shape of the spotted jewelweed, *Impatiens capensis*, an indigenous plant of North America that is a pollination generalist and that is present in urban and non-urban environments. Variation of flowers in frontal and profile views was quantified using geometric morphometrics for 228 individuals from six populations from the region of Montreal, Canada. We tested both the direct impact of urbanization (vegetation, impervious surfaces) on floral shape as well as its indirect effect via its impact on pollinator visitation rates using redundancy analyses (RDA). Results showed that urbanization significantly affects floral shape (8% of the total variation), but only through its impact on pollinator communities. These results suggest that *Impatiens capensis* could rapidly adapt to the new environments present in cities by modifying its floral phenotypes in order to increase its reproductive success.

4.2. Introduction

Plants display different floral traits to attract animals as biotic agents of pollination and to ensure proper pollen deposition on their bodies to maximize pollination: colour, floral shape, nectar or scent (Baker & Baker, 1990; Faegri & Van Der Pijl, 2013; Fenster *et al.*, 2004; Perret *et al.*, 2000). Floral shape in particular is critical for the plant reproduction because of both its role in attracting pollinators (Kaczorowski *et al.*, 2012) and in the mechanical fit between the flower and the pollinators mouthparts or bodies to ensure proper pollination (Anderson & Johnson, 2009; Maglianesi *et al.*, 2014; Muchhala, 2006; Temeles *et al.*, 2013). Floral shape can thus be the result of adaptations to specific pollinators such as a corolla tube shape adapted for hummingbird pollination (Abrahamczyk & Kessler, 2015; Castellanos *et al.*, 2004) or the presence of long spurs in hawkmoth pollination (Baker, 1961; Darwin, 1862; Kaczorowski *et al.*, 2012). These constant selective pressures by pollinators on floral shape generally result in stabilizing selection on shape

traits (Cresswell, 1998). If floral shape can be specialized to a specific pollinator, plants that are generalist in pollination also respond to selective pressures by different pollinator guilds. This leads to a combination of different floral traits, and a floral shape that is adapted to specific communities of pollinators (Dellinger, 2020; Gómez *et al.*, 2015; Joly *et al.*, 2018; Muchhala, 2007a).

Changes in plant traits can be caused, directly or indirectly, by changes in their environment. For instance, the evolution of new traits in the history of a plant family can indicate an adaptation to a new environment, such as when a lineage establishes into a new area or faces changes in biotic (Martén-Rodríguez *et al.*, 2010; Roalson & Roberts, 2016) or abiotic environments (Berry & Bjorkman, 1980; Shannon, 1997). Some examples of plant adaptation to their biotic environment, other than adaptation to pollinators, include defences against herbivory using secondary metabolites (Chew & Courtney, 1991; de Castro *et al.*, 2018; Kessler & Baldwin, 2002; War *et al.*, 2018) or mechanic defences such as spines (Young *et al.*, 2003). Numerous plant traits are also known to evolve as a result of a change in abiotic environment, such as when they colonize a new biome and, more recently, adaptation to urban environments (King & Buckney, 2000; Neil & Wu, 2006). Overall, plants have the capacity to adapt rapidly when facing changes in climate or soil composition (Jump & Peñuelas, 2005; Marschner, 1991).

In the last decades, several studies investigated the possible effects of urbanization on the evolution of plants and animals (Johnson & Munshi-South, 2017; Johnson *et al.*, 2015; McKinney, 2002; Rivkin *et al.*, 2019; Santangelo *et al.*, 2018). Urbanization can impact species via habitat fragmentation or loss, loss of nesting area, and loss of resources (McKinney, 2002; Wenzel *et al.*, 2020). Studies on plants or animals have generally found a reduction in species richness in highly urbanized areas (Cam *et al.*, 2000; Gortat *et al.*, 2014; Łopucki *et al.*, 2013; Marzluff, 2001), whereas an increase was observed where urbanization was more moderate (Dolan *et al.*, 2011; McKinney, 2008) due to the presence of species introduced by humans (McKinney, 2008; Ordeñana *et al.*, 2010; Walker *et al.*, 2009). Some impacts are indirect, such as the changes in nutrients in the sediment of urban streams that impose a strong ecological filter on species that can live under these conditions (King & Buckney, 2000).

Urbanization results in the local extinction of native species, sometimes to the benefit of non-native ones that have a greater plasticity (McKinney, 2002). However, the impacts of urbanisation are not always negative (Gregg *et al.*, 2003) as some species can become acclimatized to these new disturbed environments (Crocì *et al.*, 2008; Ordeñana *et al.*, 2010; Prange & Gehrt, 2004; Russo & Ancillotto, 2015). This concerns mainly species with a high potential of adaptation to new habitats that benefit from such changes. In contrast, native species that do not have a great adaptive ability (or suffer from competition in cities) have to relocate to new areas to avoid local extinction (Alberti *et al.*, 2017; Crocì *et al.*, 2008; Russo & Ancillotto, 2015; Stark *et al.*, 2020).

Urbanization can also impact the phenotypes of species (McKinney, 2008). In plants, increase of urbanization has been associated with earlier spring time flowering (Fisogni *et al.*, 2020; Neil & Wu, 2006) and lower flower production (Rivkin *et al.*, 2020). Overall, the rapid and often important changes imposed by cities implies a steep selective gradient, which can be mirrored by higher rates of phenotypic changes in cities compared to natural zones (Alberti *et al.*, 2017). For animal species that have the plasticity to acclimatize to urban habitats, the impacts on phenotype can be important (Alberti *et al.*, 2017), such as body-mass (Turgeon *et al.*, 2015), genetic diversity (Merckx *et al.*, 2018; Schmidt *et al.*, 2020), but also winter survival (Alberti *et al.*, 2017; Łopucki *et al.*, 2013) or health (Russo & Ancillotto, 2015). For example, it has been shown that raccoons, an opportunistic species, acclimatize well to urban environment even if their body mass is largely impacted, positively for females and negatively for males (Turgeon *et al.*, 2015).

The effect of urbanization on pollinators has also received attention (e.g., Bates *et al.*, 2011; Burdine & McCluney, 2019; Fisogni *et al.*, 2020; González-Varo *et al.*, 2013; Wenzel *et al.*, 2020). These studies highlight the fact that in addition to the potentially direct impact on the evolution of plant reproductive traits, urbanization also has the potential to indirectly affect their evolution via its impact on pollinator communities and the decline of pollination services. Urbanization has diverse impacts on pollinator species because it creates habitat fragmentation (Geslin *et al.*, 2013; Soga *et al.*, 2014), modifies floral and nesting resources (Somme *et al.*, 2016; Theodorou *et al.*, 2017), and changes abiotic variables such as temperature (Hamblin *et al.*, 2018). A review of the subject has shown that urbanization can negatively or positively influence the abundance, behaviour, phenology, foraging and nesting strategies of pollinators species, especially bees and bumblebees (Wenzel *et al.*, 2020).

The abundance, species richness and visitation rates of pollinators generally decreases with higher levels of urbanization (Bates *et al.*, 2011; Burdine & McCluney, 2019; Hamblin *et al.*, 2018; Plascencia & Philpott, 2017; Verboven, Uyttenbroeck, *et al.*, 2014; Wenzel *et al.*, 2020). Pollinator communities also differ among populations affected by different levels of urbanization (Geslin *et al.*, 2013; Larson *et al.*, 2014; Leong *et al.*, 2016; Martins *et al.*, 2017; Matteson *et al.*, 2013). It has also been shown that plants and pollinators could respond differently to urbanization, causing a desynchronization between the earlier flowering period of plants in cities and the unaffected flight period of pollinators (Fisogni *et al.*, 2020). Indeed, variation in seasonal abundance and diversity of pollinators is less important in urban areas than in natural sites (Hung *et al.*, 2017; Leong *et al.*, 2016).

If urban habitats have a strong impact on pollinator communities compared with natural sites, they often present better conditions than agricultural sites, at least at intermediate levels (as found in suburbs) of urbanization (Wenzel *et al.*, 2020). Indeed, cities have sometimes been considered a refuge for pollinators (McFrederick & LeBuhn, 2006; Samuelson *et al.*, 2018) because of the absence of agricultural pesticides, the low density of large herbivores, and the abundance of flowers provided by open habitats such parks and gardens (Carper *et al.*, 2014; Plascencia & Philpott, 2017; Quistberg *et al.*, 2016; Wray & Elle, 2015), with the addition of horticultural plants to the native ones already present (Hall *et al.*, 2017). For these reasons, cities present a greater abundance and richness of pollinators (Martins *et al.*, 2017; Quistberg *et al.*, 2016; Verboven, Uyttenbroeck, *et al.*, 2014), and increased pollination services (Potter & LeBuhn, 2015; Theodorou *et al.*, 2017; Theodorou *et al.*, 2016; Verboven, Aertsens, *et al.*, 2014), compared to agricultural lands.

These studies on pollinators communities and pollination services show the potential of urbanization to affect plant reproduction either directly or indirectly. Several studies have investigated both the direct and indirect effects of urbanization via pollinator abundance on plant phenology and phenotype (Neil *et al.*, 2014; Rivkin *et al.*, 2020; Theodorou *et al.*, 2017; Verboven, Aertsens, *et al.*, 2014; Wray & Elle, 2015), including their reproductive strategies (Barker, 2018; Rivkin *et al.*, 2020; Ushimaru *et al.*, 2014), though few have investigated the effect of urbanization on flower shape. However, the study of this trait is important because a good fit between the corolla shape and the pollinators is critical to maximize pollination effectiveness (e.g. Galen, 1989;

Muchhala, 2007b). Therefore, if floral shape cannot evolve rapidly to track changes in pollinator communities, plants could thus be maladapted to these new environments (Cornille *et al.*, 2018; Lofflin & Kephart, 2005). This is even true for plants that are generalist in pollination (Gómez *et al.*, 2009). In this study, we thus investigated the effect of urbanization on floral shape in the spotted jewelweed *Impatiens capensis* Meerb. by quantifying both its direct and indirect effects via changes in pollinator communities.

The genus *Impatiens*, and *I. capensis* in particular, have been the subject of several studies that investigated its floral variation, especially the orientation of the nectar spur (Abrahamczyk *et al.*, 2017; Rust, 1977; Travers *et al.*, 2003; Wang *et al.*, 2013; Young, 2008). *Impatiens capensis* is native to North America where it grows in damp soils, often near open water, though it can also be found in urban areas (Barker, 2018). The plant can reach 1.5 meter high. It flowers between July and mid-September. Two types of flowers are present in the species: chasmogamous (pollination by biotic vectors) flowers and cleistogamous (closed, auto-pollination) flowers that allow autonomous self-fertilization. Chasmogamous flowers are mostly present in populations that receive direct sunlight, whereas cleistogamous flowers are present in all populations but are essentially the only type of flower present in shaded populations (Waller, 1980). The chasmogamous flowers are protandrous (Rust 1979), zygomorphic, orange-yellow with red dots and they present a nectar spur on a highly modified sepal (Figure 2 A). The fruit is a capsule with explosive dehiscence. Based on the literature, the main pollinators of *Impatiens capensis* are *Bombus sp.*, *Apis mellifera*, *Archilochus colubris*, and sometimes *Vespula sp* (Rust, 1977). For this reason it can be considered a generalist species in terms of its pollination. This pollination strategy is ideal to study the indirect effect of urbanization on the species via changes in pollinator communities. Another factor that makes *I. capensis* a good model is that it is an annual plant that can potentially respond rapidly to selection (Lechowicz *et al.*, 1988). Finally, a study of flower shape in three populations from the region of Montreal suggested significant variation in floral shape among populations (Burkiewicz J. and Joly S., unpublished data). Due of the presence of this floral variation and its presence in natural and disturbed habitats, *I. capensis* seems is thus a good model to study the effect of urbanization on floral shape.

In this study, we quantified floral shape using geometric morphometrics on pictures of flowers in frontal and profile views in six populations that vary in terms of urbanization intensity. We show

that urbanization does affect floral shape of *I. capensis*, but that the effect is entirely mediated by changes in pollinator communities.

4.3. Methods

4.3.1. *Populations studied*

Six populations of *Impatiens capensis* were studied. Three were located in suburban zones and three in more natural sites (Figure 12). The suburban sites are all located in the city of Laval (noted with the prefix “SU”). One population on Rang St-François (SU-RSF) is a prairie on the side of a calm road near a highway (N45.61418° W73.70214°). The Rue Bergeron population (SU-RB) is at the extremity of a dead-end and the population starts in a flowering prairie and continues by a stream in a forest (N45.652144° W73.752036°). The last suburban population is located near the golf course UFO (SU-UFO) and is located along a path crossing a stream in a forest (N45.54913° W73.85662°). The three other populations are located in natural parks and ecological reserves (noted with the prefix “N”). One natural site is in the Oka National Park (N-OKA), in a prairie along the road crossing the park (N45.48597° W74.02457°). Another natural population is in the park Rivière-du-Nord (N-RDN) along a stream (N45.868984° W74.084186°). The last population is located along the road in a protected reserve near the Station de Biologie des Laurentides (N-SBL) of the Université de Montréal, in the town of Saint-Hippolyte (N45.974711° W74.020419°).

4.3.2. *Quantification of urbanization*

Urbanization was quantified at each site by calculating the mean NDVI (Normalized Difference Vegetation Index), the proportion of impervious surfaces, and the proportion of high and low vegetation area. All parameters were estimated from the NDVI index, a commonly used vegetation index (Esau *et al.*, 2016; Pettorelli *et al.*, 2011; Shahabi *et al.*, 2012). The non-vegetal stratum in our studied sites are essentially urban structures, allowing an estimation of impervious surfaces.

The NDVI was obtained from a Sentinel2 tile (Drusch *et al.*, 2012) that included the six sites and that was photographed on 2019-09-18, which corresponds to the blooming time of *I. capensis*. The NDVI was calculated after an atmospheric correction using the “Semi-automatic classification” plugin in QGIS vers. 3.1.2.2 using the layers 8 and 4, following the formula: $NDVI = (NIR - Red) / (NIR + Red)$.

We estimated the different variables for circles of 500m radius around the populations, as this seemed to be the best size to take account of the pollinator behaviour and the urban structure of each site. We used the following NDVI values to quantify the proportion of different surface types at each site: -0.5 to -0.1 = water, -0.1 to 0.2 = non-vegetal (= impervious surfaces), 0.2 to 0.6 = low vegetation (grass, shrubs, meadows), 0.6 to 0.8 = higher vegetation (trees, forest). The mean NDVI by site and the percentage of each surface type was calculated for each site using the plugin “r.reclass” and “r.stats” of GRASS 7.8.2, which gives the pixels number corresponding to each three classes. We validated that pixels classified as non-vegetal represented impervious surfaces in all circles around the populations. Water was removed from the estimates to better focus on the vegetation / impervious surfaces ratio.

4.3.3. Pollinator observation

At each site, pollinators were observed on two non-consecutive days, from August 8th to 30th 2019, during the blooming season of *I. capensis*. One day focused on morning pollination (8 a.m. to 2 p.m.) and the other on afternoon pollination (2 p.m. to 6 p.m.). Two persons observed a given number of open flowers and noted all visits. We confirmed whether the visitor contacted the reproductive organs (pollination), as well as the identity of the visiting species, the time of visit and the number of flowers visited by the animal. For some sites (3), a video camera was also used to record visits to additional flowers. Only pollinators were considered in the following analyses, so the visitors and nectar robbers were not taken into account. The abundance of each pollinator species was also noted, i.e. each pollinator that comes around the observed flowers. A Shannon diversity index was measured on this abundance at each site, using the ‘diversity’ function from the “vegan” package.

From these observations, we calculated the pollination rate per species, which is the number of visits of a pollinator species per flower, per hour. To illustrate the differences in pollinator visits among the populations, we performed a principal component analysis of the Hellinger-transformed visitation rate matrix (Legendre & Gallagher, 2001).

4.3.4. *Flower shape data and morphometry*

One flower from 40 individuals per population (20 in Golf UFO due to its smaller size) was photographed in frontal and profile views (Figure 13 A, B) with a ruler to account for size. Two pictures of each flower in the two positions were taken and processed independently to quantify the technical errors involved in the photography and morphometric approaches. We noted the developmental stage of the flower, which was either “pre-anthesis”, “male”, “female” or “fruit”, to take it into account in the analyses.

We used geometric morphometrics to study the flower shape. Profile photos were digitized using the TpsDig software (Rohlf, 2004). Six landmarks (homologous points) were used: one at the tip of the nectar spur, one at the point connecting the flower to the pedicel, one at the tip of the top petal, one at the top of the curve formed by the basal petal (semi-homologue point), and one at the tip of the lower petals (Figure 13 A). Two curves were used on the profile view: one follows the curve of the sepal from the point where it is attached to the pedicel to the tip of the spur, and another along the bottom part of the sepal from the tip of the spur to the end of the sepal. Each curve was quantified 20 semi-landmarks.

The frontal photographs were digitized with ImageJ (Rasband, 1997) using 8 landmarks and 6 curves (Figure 13 B). The landmarks were positioned at the tip of the standard (top) petal, at the intersection between the standard and wing petals, at the intersection between the wing and the standard petals, at the intersection between the two standard petals, and the two widest points of the flower tube opening. Two curves described the shape of the standard petal (8 semi-landmarks each), two to quantify the shape of the side of the flower formed by the lateral petals (wings) (4 semi-landmarks each), and two curves of 14 semi-landmarks each to describe the shape of the bottom petals.

We performed a generalized Procrustes analysis with the geomorph R package (Adams *et al.*, 2016) in R (R core team 2014) to superimpose all digitized flowers for further analyses. The semi-landmarks were superimposed by minimizing the Procrustes distance between the reference and the target flower shape in frontal photos. In profile view, the huge variation in spur orientation caused alignment problems if we allowed the semi-landmarks to slide during the Procrustes analysis. We thus treated them as fixed landmarks. Note that this does not affect our conclusions as treating them as semi-landmarks did not change the outcome of the statistical tests (data not shown).

To analyze the frontal and profile shapes simultaneously and thus have a better idea of floral variation in 3D, the mean 2D coordinates of each individual, frontal and profile, were combined in a single 3D array, keeping the two sets of coordinates orthogonal (van de Kerke *et al.*, 2020). To combine the datasets, a uniform z coordinate was added to all pictures in profile view (i.e., the original x coordinate of the point at the tip of the standard petal for pictures in frontal view); the x coordinates of the frontal pictures became z coordinates and the frontal pictures were attributed a uniform x coordinate (i.e., the x coordinate of the point connecting the flower to the pedicel in profile view). The two datasets were combined and the resulting 3D matrix was analysed by principal component analysis (PCA) of the covariance matrix using the geomorph ‘gm.prcomp’ function.

4.3.5. Statistical analyses

A Procrustes ANOVA was performed on the combined floral shape data to partition the variability in flower shape between individuals and populations. The ANOVA was performed using the ‘procD.lm’ function of the package “geomorph”.

The effect of pollinators visitation rates, urbanization and floral developmental stage on flower shape was tested by redundancy analyses (RDA) using the “vegan” package. The response matrix consisted of the significant principal components of the flower shape as determined using the Brokenstick criteria (“vegan” package, (Oksanen *et al.*, 2007)). The pollinator data was the Hellinger transformed rate visitation matrix to avoid considering the shared absence of pollinators

as evidence of similarity (function “decostand” of “vegan” package, Oksanen *et al.*, 2013) . The urbanization matrix consisted of the mean NDVI and the proportion of impervious surfaces, low vegetation, and high vegetation within a circle of 500 m radius around each site. Urbanization, pollinator visitation rates and developmental stages were analysed independently and simultaneously. The partitioning of the total shape variation according to the independent matrices was done with the “varpart” function of the “vegan” package and the significance was tested by partial RDA. An additional RDA was done to study the effect of urbanization on the pollinators visitation rate.

4.4. Results

4.4.1. Quantification of urbanization

We calculated the mean NDVI, the percentage of no vegetation, low vegetation and high vegetation in a 500m radius circle around each site (Table 7). The three natural sites had a much higher mean NDVI, as the zone show a high proportion of high vegetation. The three suburban sites had a greater proportion of impervious surfaces and a greater proportion of low vegetation.

4.4.2. Visitors and pollinators observed

We totalled 107.5 hours of observation, which corresponds to 3883 hours of observation per flower when considering that several flowers were observed simultaneously. Fifteen species (or genera in some cases) of visitors were observed: *Archilochus colubris*, *Bombus impatiens*, *Bombus vagans*, *Bombus ternarius*, *Vespula sp.*, *Anthidium florentinum*, *Ocyptamus fascipennis*, *Syrphus sp.*, *Rhingia nasica*, *Toxomerus geminatus*, *Papilio cresphontes*, *Augochlorella aurata*, *Andrena sp.*, *Xylocopa sp.* and *Sceliphron caementarium* (cf Table S1 for common names). Because *Bombus vagans* and *B. impatiens* were difficult to distinguish in the field, we combined them into a single *Bomops sp.* category. We were able to identify *Bombus ternarius* with its distinctive orange band on the abdomen.

Species were considered pollinators when they made contact regularly with the reproductive organs. The most frequent pollinators were the ruby-throated hummingbird (*Archilochus colubris*), the common eastern bumblebee (*Bombus impatiens*), the half-black bumblebee (*Bombus vagans*) and the western honeybee *Apis mellifera* (Figure 14). Wasps from the genus *Vespula* (*Vespula* sp.), the orange belted bumblebee (*Bombus ternarius*), and *Anthidium florentinum* - one of the European wool carder bees - were also pollinators but were less frequently observed. The other species observed entering the flowers did not make contact with the reproductive organs and were considered as non-pollinators. The flower populations with the greatest abundance of pollinators were not necessarily the ones with the greatest diversity. The populations RB, SBL and RSF had the highest Shannon diversity index (1.01, 0.90 and 0.78, respectively), whereas OKA, RDN and UFO were found to have a lower diversity (0.20, 0.22 and 0.26).

4.4.3. Pollinator visitation rate

We calculated the visitation rate of animal visitors and pollinators, which is the number of visits by a specific species per flower per hour (Supplementary Table S1). A PCA of the pollinator visitation rate data illustrates how sites differ in terms of pollinators (Figure 15). For instance, UFO, RDN, and OKA have a high *Bombus* sp. visitation rate, SBL and RSF have a high visitation rates of *Apis mellifera*, and OKA and RB are characterized by more frequent visits of *Archilochus colubris*.

Urbanization was found to have a strong impact on pollinator visitation rates ($\text{adj}R^2=0.774$, $p<0.001$; Figure 16). The visitation rate of *Anthidium florentinum* was positively correlated to the proportion of impervious surfaces and negatively correlated to the mean NDVI (Figure 16). The opposite pattern was found for the visitation rate of *Vespula* sp. The visitation rate of *Bombus* sp. and *Archilochus colubris* are positively correlated – even if slightly so – to the proportion of low vegetation at the sites, whereas the visitation rate of *Bombus ternarius* is negatively correlated to the proportion of low vegetation.

4.4.4. *Variation of floral shape*

According to the Brokenstick criteria, ten principal components were found to explain a significant portion of the flower shape variation. Together, these components explain 84% of the flower shape variation. The first principal component represents 24% of the shape variance and shows variation in the position of the bottom petals (more recurved to the left), in the length of the spur (longer to the left) and in the shape of the bottom petals (rounder to the right; Figure 17). The second principal component explains 18% of the variance and is characterized by the curvature of the spur (curved spur is associated with positive values) and by the presence or absence of a space between the two bottom petals (gap associated with negative scores; Figure 17). Variation in the first five axes is presented in Figure 18.

The Procrustes ANOVA showed significant differences in shape between the different populations ($R^2=0.095$; $p\text{-value}<0.001$; Figure 17) even if most of the variation (90%) occurs among individuals in populations.

4.4.5. *Effect of pollinators and urbanization on floral shape*

Partition of the floral shape variance between pollinator visitation rate, the urbanization and the developmental stage of the flowers by means of RDA analyses shows that the developmental stage explained 5% ($p\text{-value}<0.001$) of the variance and it is completely independent from the variance explained by the urbanization and pollinators and thus do not affect the results (Supplementary Figure S1).

The pollinator visitation rate and the urbanization together explain 8.2% of the floral shape variation in the RDA ($\text{adj}R^2=0.082$, $p<0.001$). Of this variation, 6.9% is co-explained by both variables (Figure 19), and 1.3% is explained by only the pollinator visitation rates ($\text{adj}R^2=0.013$, $p<0.001$). In contrast, no significant fraction of the variation can be explained solely by the urbanization information ($\text{adj}R^2=0$).

The constrained ordination obtained with the RDA including pollinator visitation rate and urbanization in scaling 2 illustrates correlations between floral shape components and explanatory variables (Figure 20). In particular, it shows that positive scores on PC2 for floral shape are positively correlated to the mean NDVI and negatively correlated with the proportion of impervious surfaces and low vegetation (Figure 20). Positive scores on PC4 have a relatively strong positive correlation with the proportion of impervious surfaces and finally positive scores on PC3 have a positive correlation with *Apis mellifera* and a negative correlation with *Bombus* sp.

4.5. Discussion

In this study, we used geometric morphometrics to test the hypothesis that urbanization affects floral shape in *Impatiens capensis*. Our results showed that urbanization impacted the flower shape of *I. capensis*, but that the effect is entirely indirect. Indeed, it is the changes in pollinator guilds, largely affected by urbanization, that explains changes in flower shape (8% of variance explained). Previous studies have also reported the indirect effect of urbanization via pollinators on plant phenology, seed production and reproductive strategies (Neil *et al.*, 2014; Rivkin *et al.*, 2020; Theodorou *et al.*, 2017; Ushimaru *et al.*, 2014; Verboven, Aertsen, *et al.*, 2014; Wray & Elle, 2015). However, to our knowledge, this is the first evidence of an effect on floral shape. With the use of geometric morphometrics and the combination of frontal and profile pictures of flowers, we were able to precisely quantify and describe the effect of urbanization on floral shape.

The effect of urbanization on pollinator communities was most evident at the level of the community assemblages between the sites, as observed in previous studies for other species (Geslin *et al.*, 2013; Larson *et al.*, 2014; Leong *et al.*, 2016; Martins *et al.*, 2017; Matteson *et al.*, 2013). We found that the visitation rate of different pollinators was affected by the urbanization level at each site. *Bombus* sp. and *Archilochus colubris* were, even if slightly, associated with low vegetation, and *Bombus ternarius* was negatively linked to this variable (Figure 5). *Anthidium florentinum* was associated with high levels of impervious surfaces, at the opposite of *Vespula* sp. In contrast, we did not find a linear relationship between urbanization and either pollinator abundance or diversity. *Bombus* sp. was the most abundant pollinator in most of the sites, even in

more urban areas, which is consistent with others studies that observed an abundance of bumblebees in cities (McFrederick & LeBuhn, 2006; Theodorou *et al.*, 2016; Verboven, Aertsen, *et al.*, 2014). The second most abundant pollinator was *Apis mellifera*, whereas the other pollinators were never abundant at any site. In terms of diversity, we also did not find a correlation with urbanization. Two populations from the suburban sites were also among the most diversified populations. This supports other studies that found high pollinator diversity in urban sites (Martins *et al.*, 2017; Quistberg *et al.*, 2016; Verboven, Uyttenbroeck, *et al.*, 2014). This also suggests that urban sprawl could provide enough floral resources and nesting habitat for several pollinator species (Theodorou *et al.*, 2017).

Our results provide some information as to which flower shapes are associated with different pollinators. For example, the constrained ordination of the RDA (Figure 9) showed that the floral shapes characterised by high scores on the third principal component (PC) and to a lesser extent to high scores on the fourth PC are positively correlated to the visitation rate of *Apis mellifera* and negatively correlated to the visitation rate of *Bombus sp.* *Apis mellifera* is thus more frequent in populations in which flowers have long and non-curved spurs and compressed standard petals, whereas *Bombus sp.* is more associated with flowers with a short curved spur and a large and wide standard petal. These results could imply that those shapes are favoured by those pollinators although further studies, especially other cities, are needed to confirm these correlations. Previous studies have shown that some flower shape traits are associated with pollination syndromes in *Impatiens*, such as the length of the sepal carrying the spur or the flower size (Abrahamczyk *et al.*, 2017). This further supports the idea that some shapes may be favoured by particular pollinators, even in a pollination generalist species. Other floral traits are known to be under the selection of pollinators in this family, such as nectar volume and sucrose proportion (Abrahamczyk *et al.*, 2017; Vandeloek *et al.*, 2019), and as such it would be interesting to study these in an urbanization context in the future.

Even if the urbanization level was not found to have a direct impact on the floral shape of species, the constrained RDA ordination was interesting as it suggested that some floral shapes were highly correlated with some aspects of urbanization. Impervious surfaces and mean NDVI had vectors pointing in opposite directions in the constrained ordination (Figure 9) and they were found to be positively correlated to the second and fourth principal components, respectively. A higher mean

NDVI is thus associated to flowers with closer bottom petals and a large and open standard petal whereas higher impervious surfaces are associated with the opposite, that is flowers with more separated bottom petals and a more compressed standard petal. Despite the fact that they did not study floral shape, Barker & Sargent (2020) suggested that greater urbanization decreases seed set, even if it doesn't affect pollen limitation.

Our results suggest that *I. capensis* can adapt to changes in its biotic (pollinator) environment, but this is likely facilitated in this species because of its pollination strategy. *Impatiens capensis* is a pollination generalist and thus can be pollinated by several insect pollinators as well as hummingbirds. Therefore, a generalist species is much more likely to survive the loss of one pollinator in a given population than a pollination specialist. This suggests that pollination generalists are better able to face changes in their biotic niche because they often do not have to drastically change their phenotypes to ensure their reproduction. Pollination generalists have often been associated with habitats characterized by poor pollinator density and diversity (Armbruster & Baldwin, 1998; Barrett *et al.*, 1996; Olesen & Jordano, 2002; Waser, 2006), and as such this should also be a winning strategy to cope with environmental changes such as the spread of urbanization. Furthermore, our results suggest that in addition to being able to be pollinated by a range of pollinators, *I. capensis* also adapts to changes in its pollinator communities via modifications in its floral shape. Similar floral shape adaptations have been documented for other pollination generalists in the past (Gómez *et al.*, 2015), even for extreme generalist species that have specific shapes associated with particular pollinator guilds (Gómez *et al.*, 2016). *I. capensis* is another example of an indigenous species that can rapidly adapt to changes in the environment (Gregg *et al.*, 2003; Johnson *et al.*, 2015; Ushimaru *et al.*, 2014; Verboven, Aertsens, *et al.*, 2014).

In brief, this study shows that urbanization can affect the evolution of floral shape, albeit indirectly by affecting the composition of the pollinator communities. While previous studies have tested the indirect effect of urbanization on floral phenology and reproductive strategies via its impact on pollinator communities, we show here that urbanization can also affect the floral shape of angiosperms. Future studies of the impact of urbanization on floral shape could lead to a better understanding of the effect of urbanization on the evolution of indigenous species in these environments.

4.6. Acknowledgements

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Table 7. Mean NDVI and proportion of each type of habitat within a 500m radius circle around each population. Water was excluded of all estimates. Natural sites are identified with the prefix “N” and suburban sites are noted with the prefix “SU”.

Site	Mean NDVI	Proportion of impervious surfaces (no vegetation)	Proportion of low vegetation	Proportion of high vegetation
SU-RSF	0.48	18.00	41.84	40.16
SU-RB	0.56	7.37	40.00	52.63
SU-UFO	0.52	12.59	41.12	46.29
N-SBL	0.72	0.30	7.86	91.84
N-OKA	0.69	0.01	23.76	76.23
N-RDN	0.61	4.63	32.26	63.11

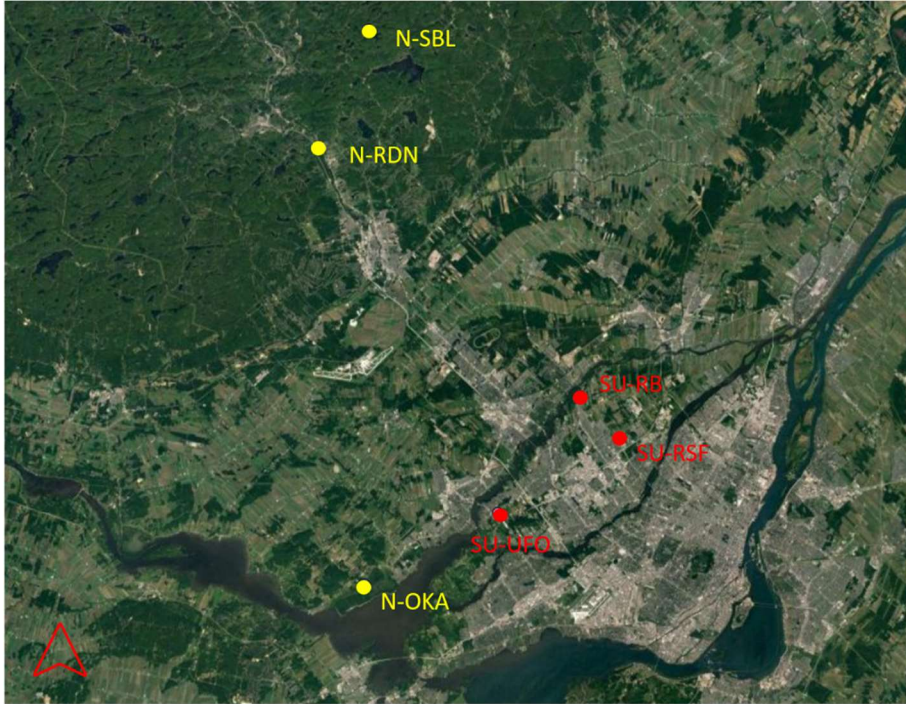


Figure 12. Map showing the studied populations in the region of Montreal, Quebec, Canada (Google maps satellite image). Natural sites are in yellow, whereas suburban sites are in red.



Figure 13. Position of the landmarks and semi-landmarks on profile and frontal pictures. Landmarks are represented by red dots and semi-landmarks and curves by the blue lines.

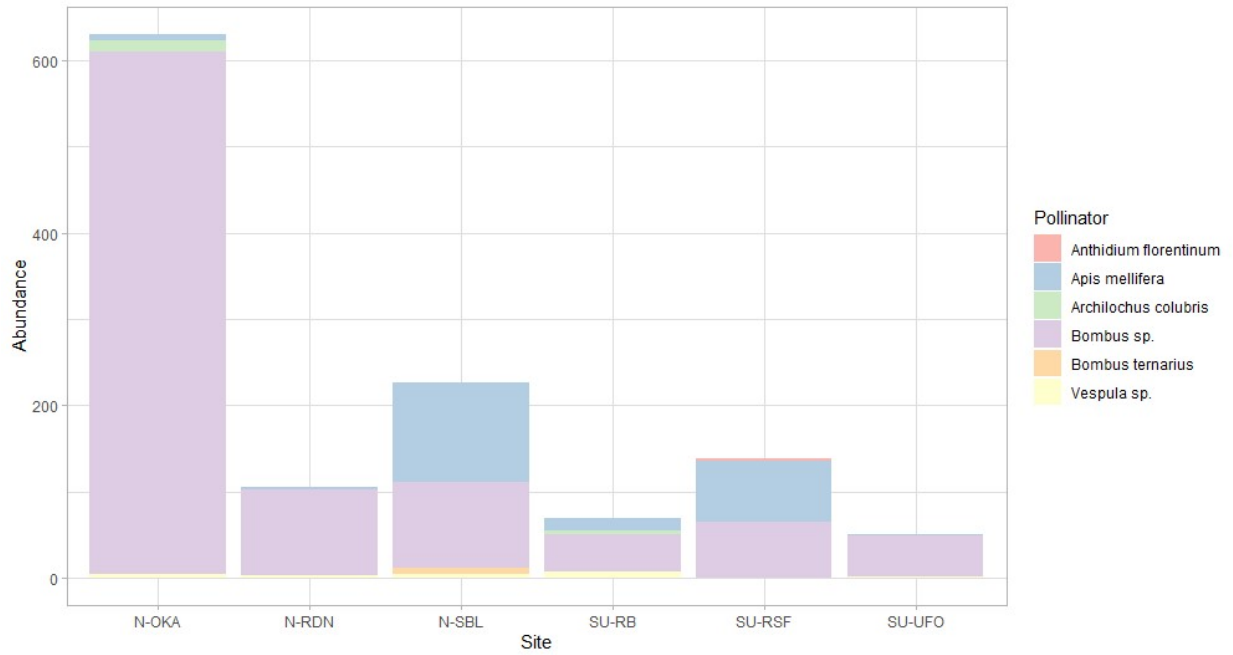


Figure 14. Histogram of the abundance of each pollinator by site.

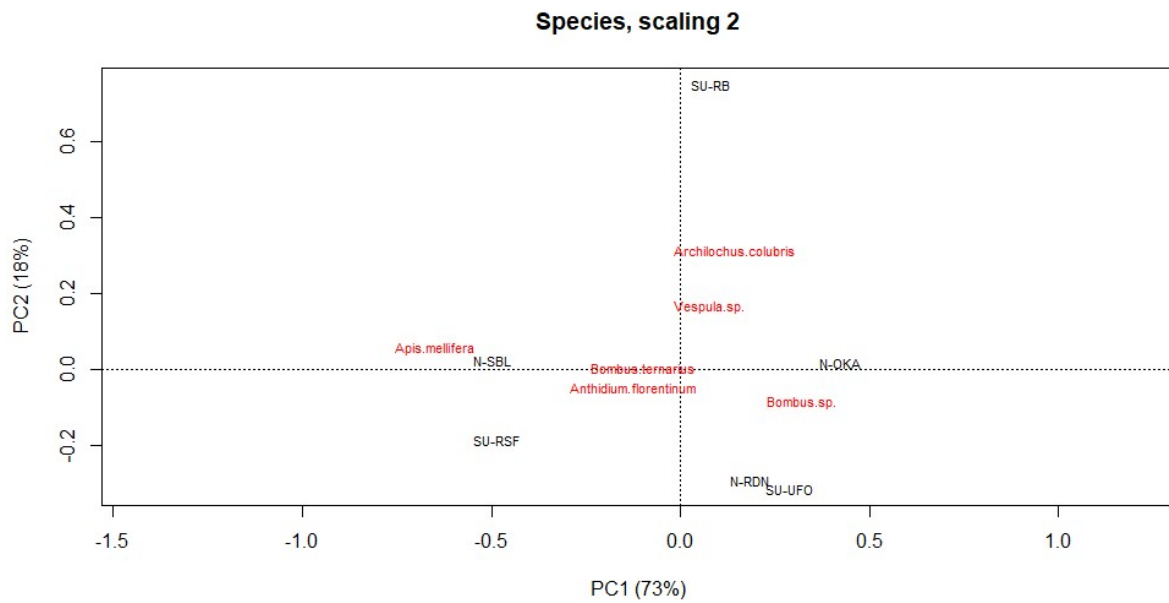


Figure 15. PCA of the visitation rates of pollinators of *Impatiens capensis* observed at the studied sites.

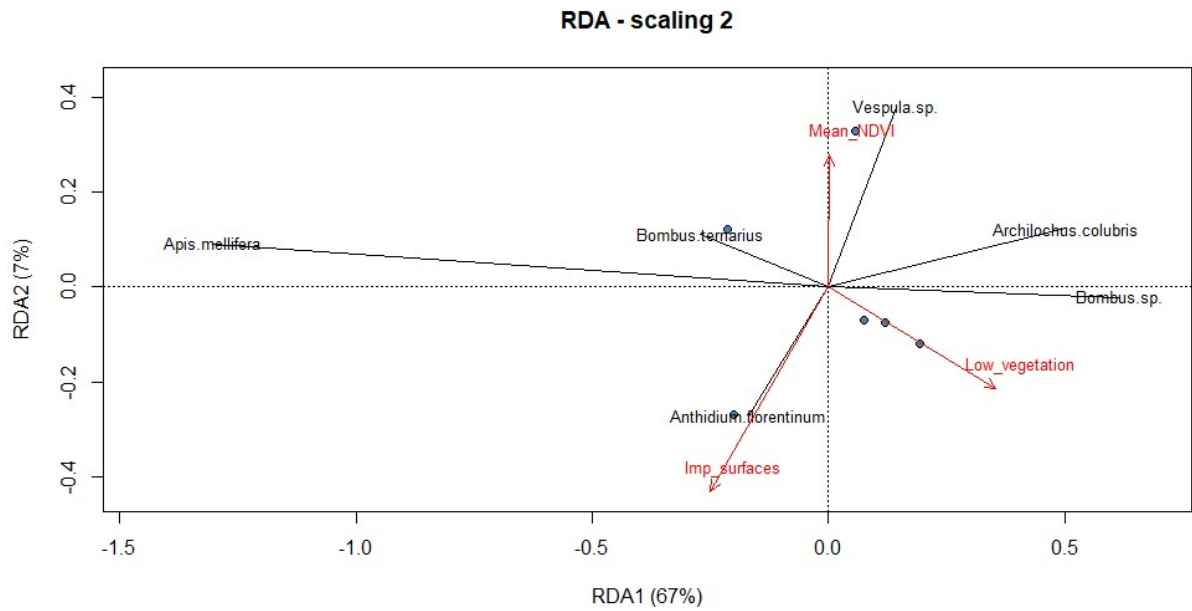


Figure 16. Triplot of the RDA testing the effect of urbanization on pollinator visitation rate.

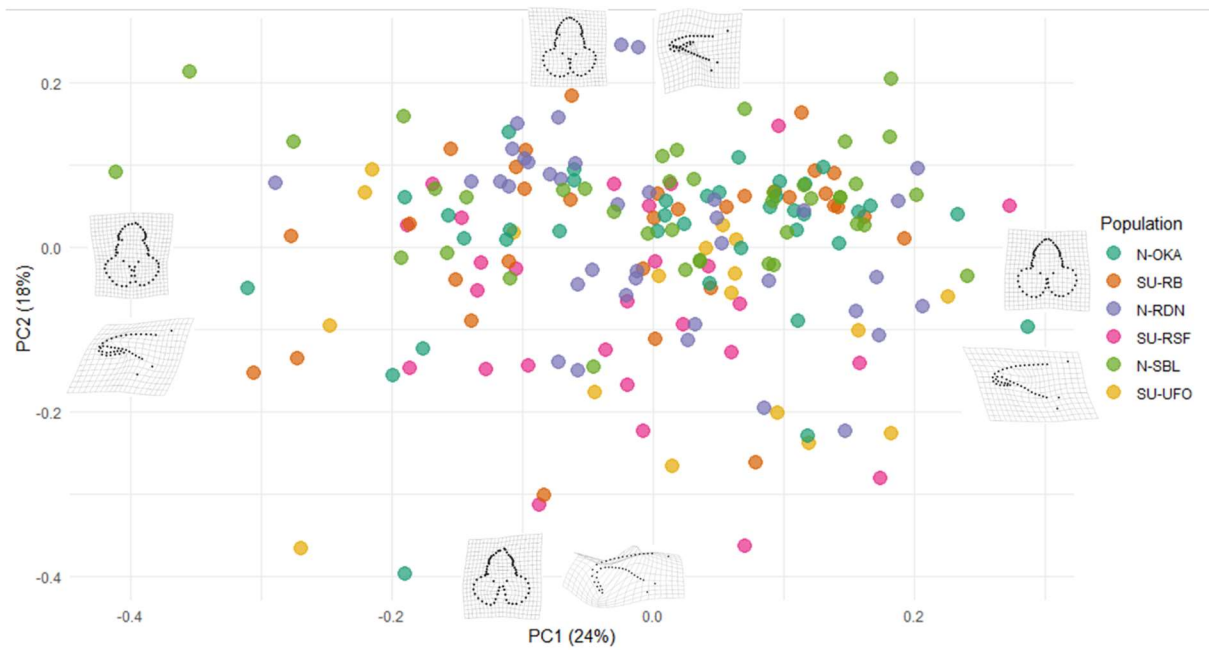


Figure 17. PCA of the floral variation between sites on the 2 first axis. Morphological variation along the axes is presented by showing the extreme floral shapes observed along each axis.

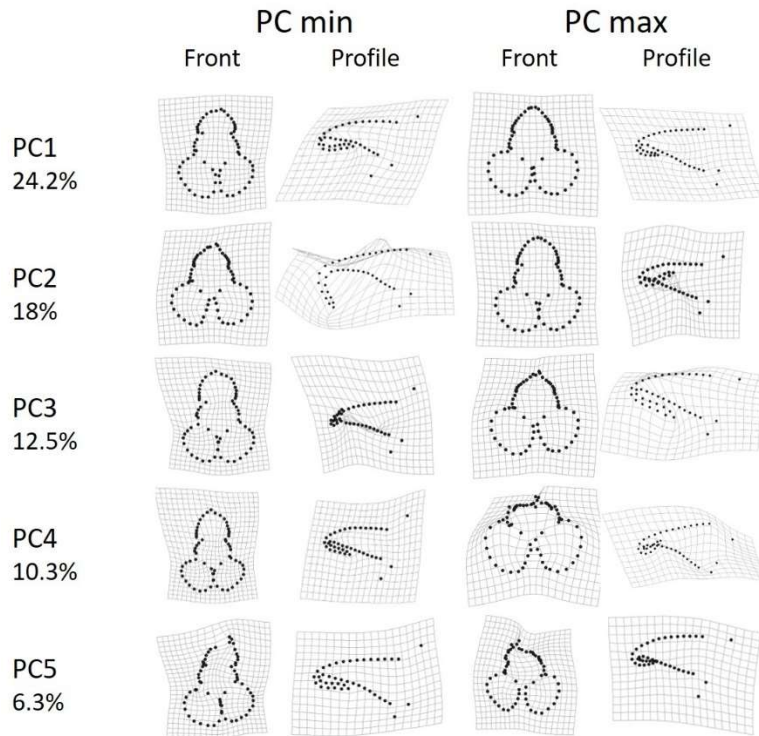


Figure 18. Flower shape variation along the first five principal components of the PCA. For each principal component, the portion of variation represented by the component is indicated and the maximum and minimum shape along each component are represented.

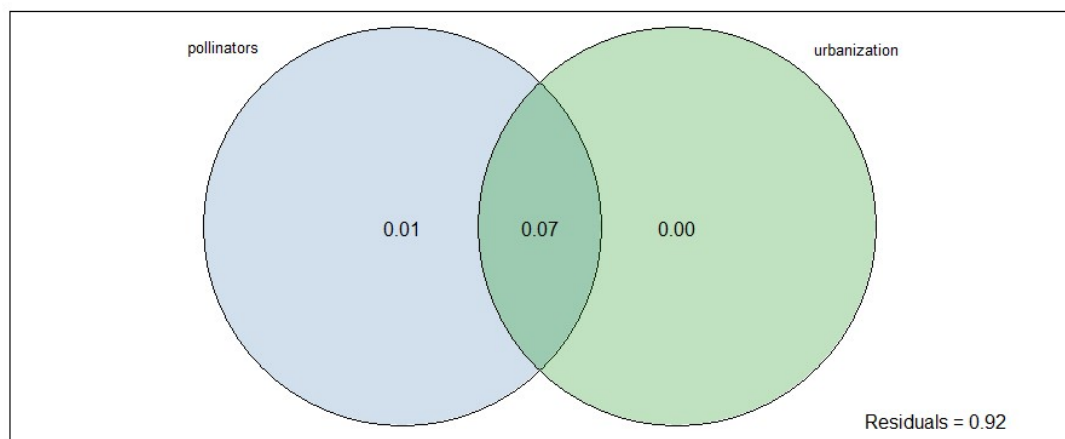


Figure 19. Venn diagram of the variance partitioning between the pollinator visitation rate and the urbanisation.

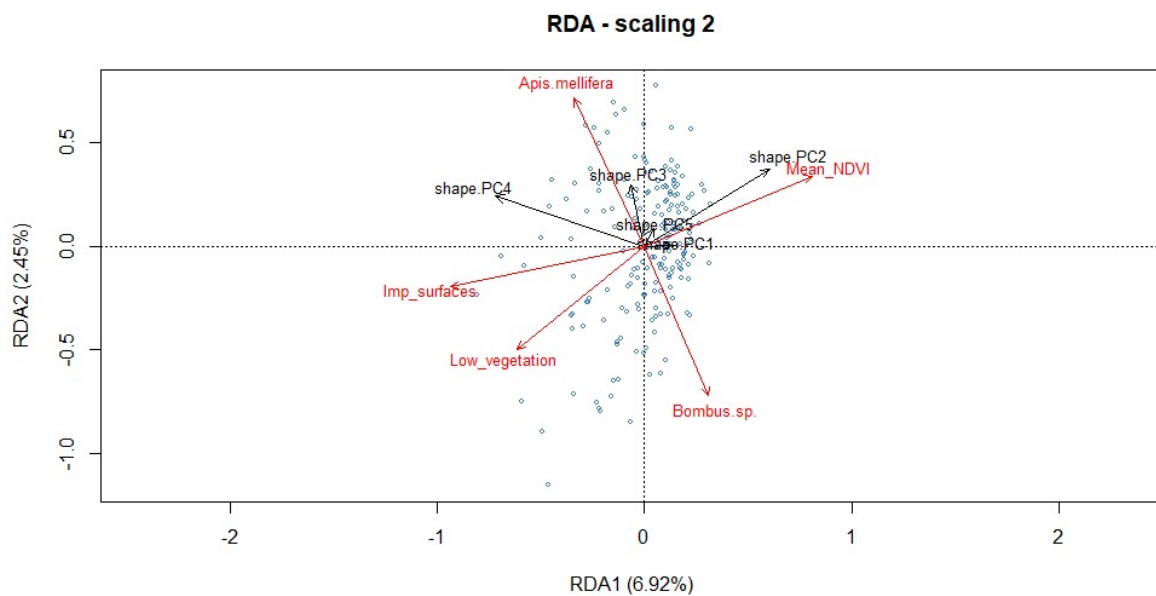


Figure 20. Triplot of the RDA testing the effect of pollinator visitation rate and urbanization on floral shape. Redundant variables are not shown on the ordination.

Chapitre 5 : Discussion

Les différentes études menées dans cette thèse ont permis d'étudier l'effet de l'environnement sur la forme florale, à travers différentes pressions de sélection : les pollinisateurs, l'insularité, et l'urbanisation. Cette thèse a ainsi pu démontrer, à différentes échelles et pour différentes espèces, que l'adaptation florale peut être la résultante de facteurs variés qui peuvent avoir une influence plus ou moins importante et parfois combinée.

5.1. Résultats principaux

La première étude cible l'effet des colibris sur la forme florale, pour des espèces spécialistes et généralistes. Les résultats de cette étude ont montré que la forme florale était corrélée à la forme du bec des colibris pollinisateurs chez les Gesneriaceae antillaises spécialisées à la pollinisation par ces oiseaux. Avec un échantillon de 18 espèces de plantes, nous avons démontré que la longueur de la corolle des fleurs est corrélée à la longueur des becs des pollinisateurs, mais seulement pour les espèces spécialistes des colibris. En rajoutant à ces mesures l'utilisation de la morphométrie géométrique, nous avons aussi montré que les formes de profil des corolles étaient corrélées aux formes de profil des becs des oiseaux. La famille des Gesneriaceae est représentée par 90 espèces dans les Antilles (toutes stratégies de pollinisation confondues). Notre étude apporte de premiers éléments d'étude sur la comparaison entre la corolle et le bec des pollinisateurs dans les îles, mais il faudrait faire les mêmes analyses sur les espèces de Gesneriinae pollinisées par les colibris manquant dans cette étude, pour que les résultats soient pleinement représentatifs de cette famille. Bien que notre échantillonnage de plantes soit réduit par rapport au nombre d'espèces de Gesneriinae se trouvant dans les Antilles, nos résultats sont pertinents et apportent des précisions concernant la littérature déjà existante sur le sujet, en montrant une adaptation forte des formes des fleurs pour les pollinisateurs (Maglianesi *et al.*, 2014; Temeles *et al.*, 2009). La relation entre la forme florale et la forme du bec était cependant faible pour les espèces généralistes en pollinisation, ce qui pourrait être expliqué par la sélection imposée par leurs autres pollinisateurs (chauves-souris et insectes). Il a été noté dans cette étude que la taille de la corolle est très variable pour certaines espèces, entre les populations, qui pourrait aussi expliquer la faible significativité pour certains de nos résultats. En effet, nous avons remarqué en mesurant les corolles, que pour certaines espèces, la taille était très variable en fonction de la source utilisée (spécimen vivant, différents herbiers, photos). C'est par exemple le cas pour *Gesneria acaulis* ou *Rhytidophyllum vernicosum*, dont nos

mesures présentait des variations par rapport aux mesures réalisées par Martén-Rodríguez *et al.* (2009). L'introduction de l'usage de la morphométrie géométrique pour ce type d'étude est avantageuse, puisque cette technique permet de prendre en compte plusieurs mesures en un seul ensemble. Il serait intéressant que de futures études sur la comparaison fleur-pollinisateur continuent d'utiliser cette approche.

La deuxième étude met de l'avant la capacité d'une espèce généraliste à survivre face à l'absence d'un de ses pollinisateurs et l'intérêt pour une espèce de plante d'être généraliste au niveau de la pollinisation dans un environnement où les populations de pollinisateurs fluctuent et/ou sont à risque, tels que les îles. Nous savons maintenant que l'espèce étudiée, *Rhytidophyllum bicolor*, est pollinisée par trois groupes fonctionnels de pollinisateurs : abeille, chauve-souris et colibris. Selon nos résultats la quantité de pollen déposée par les chauves-souris et les abeilles semble être suffisante pour assurer le succès reproducteur de la plante, alors que les colibris, pollinisateurs importants, étaient absents. Un an après le passage de l'ouragan Matthew, les populations de colibris étaient quasi-absentes, et cette absence aurait pu avoir un impact considérable sur *R. bicolor*. Étant une espèce généraliste, et les autres pollinisateurs ayant été moins touchés par l'ouragan, la pollinisation et le cycle reproducteur de cette espèce n'ont pas été stoppés. Bien que de précédentes études avaient déjà émis l'hypothèse que la stratégie de pollinisation généraliste pouvait être utile dans les environnements où les populations de pollinisateurs sont moins riches ou fluctuent (Armbruster & Baldwin, 1998; Mayfield *et al.*, 2001; Olesen & Jordano, 2002; Waser, 2006; Waser *et al.*, 1996), les résultats de cette étude valident cette hypothèse. Ce chapitre pourrait également apporter un élément de réponse au fait que les espèces de Gesneriaceae généralistes en pollinisation ne se trouvent que sur les îles des Antilles (Martén-Rodríguez *et al.*, 2009). En effet, si la stratégie de pollinisation généraliste est une adaptation mise en place par les plantes pour toujours avoir des pollinisateurs « de secours » dans des environnements où ceux-ci peuvent être menacés par les désastres naturels (ouragan, séisme, tsunami, etc.), alors c'est probablement une raison de l'existence de cette stratégie dans les îles des Antilles. Cependant, comme cette stratégie est également présente sur le continent pour d'autres familles de plantes, tels que les genres *Centropogon* et *Burmeistera* (Lagomarsino & Muchhala, 2019; Muchhala, 2003), alors il ne s'agit pas de la seule explication à la présence d'espèces généralistes uniquement sur les îles chez les Gesneriaceae. Cela pourrait être dû à plusieurs autres raisons (histoire biogéographique, colonisation des îles, communautés de pollinisateurs moins riches), qui combinées ensemble, expliqueraient ce phénomène.

La troisième étude de cette thèse explore l'effet de l'urbanisation sur la forme florale chez *Impatiens capensis*, espèce indigène d'Amérique du Nord bien adaptée au milieu urbain. En comparant la forme florale de face et profil combinée sur six sites à différents degrés d'urbanisation et de recouvrement végétal, nous avons pu montrer que le degré d'urbanisation avait un effet sur la forme florale pour cette espèce, mais seulement indirectement via des changements dans les communautés de pollinisateurs. Les résultats montrent que les taux de visite des pollinisateurs ont un effet notable sur la forme florale. Les analyses réalisées ont permis de montrer que deux pollinisateurs principaux, *Bombus sp.* et *Apis mellifera*, avaient un effet sur la forme florale, favorisant chacun un type de forme différente. Il est possible que leur taille différente, ou leur approche en vol avec la fleur, favorise certains traits floraux. La forme florale pouvant servir aussi à attirer les pollinisateurs, la variation de forme florale observée pourrait être expliquée par les pollinisateurs présents et disponibles dans chaque site. Si l'urbanisation n'impacte pas directement la forme florale chez *Impatiens capensis*, elle a un effet sur les communautés de pollinisateurs, puisque nos résultats ont montré des différences de pollinisateurs présents entre les sites, et que certains pollinisateurs seraient favorisés, ou défavorisés, par des variables urbaines. Cependant, le pollinisateur le plus abondant était toujours *Bombus sp.* dans chaque site. La composition des communautés de pollinisateurs et leur taux de visite étant impactés par l'urbanisation (Bates *et al.*, 2011; Wenzel *et al.*, 2020), il est possible d'en déduire que l'urbanisation a un effet indirect sur la forme florale chez *Impatiens capensis*, via les pollinisateurs. Il est à noter que les mesures de richesse spécifique ont montré que les sites les plus urbains n'avaient pas une valeur de richesse plus basse que les sites plus naturels. Également, les sites les plus urbains que nous avons échantillonnés se situent en banlieue urbaine. Bien que le taux d'urbanisation y soit haut, les banlieues présentent beaucoup plus d'espaces verts que les centres urbains, et les jardins apportent beaucoup de ressources aux pollinisateurs, grâce à la présence d'un grand choix de plantes horticoles en supplément des plantes indigènes (Theodorou *et al.*, 2017). La richesse en pollinisateurs de ce type de milieu n'est donc pas forcément plus réduite qu'en milieu plus naturel, et elle peut même parfois être plus haute (Dolan *et al.*, 2011; McKinney, 2008). Notre étude a été réalisée sur six sites de la région de Montréal, avec des taux d'urbanisation différents. Bien que cet échantillonnage soit suffisant pour obtenir des résultats intéressants, ces derniers auraient été plus robustes si nous avions réalisé l'étude sur un plus grand nombre de sites. Il serait nécessaire dans de futures études d'échantillonner plusieurs sites dans différentes villes d'Amérique du Nord, et de comparer si l'effet de l'urbanisation y est similaire.

5.2. Nouveaux apports pour la recherche

L'utilisation de la morphométrie géométrique est novatrice, comme discutée précédemment, puisqu'elle permet de prendre en compte des données plus précises de la forme florale, sur des photographies de fleurs de profil ou de face, permettant d'obtenir une forme très précise de la corolle. Cependant, l'utilisation de photographies de face ou de profil ne permet qu'une représentation sur deux dimensions de la forme florale. C'est pour cette raison que dans le chapitre 4, les formes florales de profil et de face ont été associées, afin de prendre en compte plus de détails de la forme florale totale. Bien que ces données associées donnent une idée plus réaliste de la forme florale, plusieurs détails sont manquants, notamment sur les parties non visibles sur les photographies de face et de profil. Afin de palier à ce manque d'informations, la méthodologie la plus complète pour avoir des données les plus précises possible de forme florale serait d'avoir les données de morphométrie géométrique sur une image en trois dimensions de la fleur, tels qu'utilisé dans de précédentes études (Dellinger *et al.*, 2019; Hsu *et al.*, 2020; van de Kerke *et al.*, 2020). Par exemple, une méthodologie a été développée par Lemenager *et al.* (in prep.) qui fait suite aux travaux de Dan Schoen (unpublished), pour obtenir la représentation 3D de la corolle par photogrammétrie. Les données de morphométrie géométrique ainsi obtenues permettent une représentation très précise de la forme florale étudiée. Avec ce type de données, la comparaison entre les pièces buccales des pollinisateurs et la forme florale pourrait être plus précise qu'avec les données 2D, et les résultats obtenus pourraient être doublement validés.

Comme il a été déjà discuté, la deuxième étude de cette thèse a permis de démontrer un avantage à être généraliste au niveau de la pollinisation pour les plantes présentes sur les îles fréquemment touchées par les perturbations naturelles. Plusieurs études ont proposés que le syndrome généraliste pouvait être utile à une plante, notamment lorsque celle-ci se trouve dans un milieu pauvre en pollinisateurs, ou sujets à des fluctuations temporelles (Armbruster & Baldwin, 1998; Martén-Rodríguez *et al.*, 2009; Olesen & Jordano, 2002; Waser, 2006; Waser *et al.*, 1996). Avec notre étude, nous pouvons valider cette hypothèse, puisque nous avons démontré que chez *Rhytidophyllum bicolor* le succès reproducteur était toujours assuré par certains pollinisateurs, malgré le fait que d'autres (i.e. les colibris) soient absents. Bien que la réalisation de futures études similaires sur d'autres espèces généralistes en pollinisation soient nécessaires pour valider de façon

plus large cette hypothèse, notre étude apporte de premiers éléments de réponse à la présence de la stratégie de pollinisation généraliste et ses avantages. Notre étude est aussi la première à quantifier l'efficacité des pollinisateurs après une visite et à calculer la performance de pollinisation pour une espèce de la famille des Gesneriaceae antillaises. Cette information est primordiale, et apporte de nouveaux éléments qui permettent de mieux comprendre l'évolution des stratégies de pollinisation dans cette famille.

L'apport majeur du quatrième chapitre est l'étude de l'effet indirect de l'urbanisation sur la forme florale. Alors que plusieurs études avaient étudié l'effet direct de l'urbanisation sur la biologie des plantes (Alberti *et al.*, 2017; Fisogni *et al.*, 2020; Neil & Wu, 2006), quelques études seulement s'étaient penchées sur les effets indirects de cette pression anthropique sur la production de graines ou encore la floraison (Barker, 2018; Neil *et al.*, 2014; Rivkin *et al.*, 2020; Theodorou *et al.*, 2020; Wray & Elle, 2015). En montrant ici l'effet des pollinisateurs sur la forme de la corolle, à travers un gradient d'urbanisation, nous avons pu montrer que l'urbanisation affecte les communautés de pollinisateurs présentes sur un site suivant le degré de surfaces imperméables présentes. Cette différence dans les communautés de pollinisateurs va avoir un effet sur la forme florale, puisque nos résultats indiquent qu'un type de pollinisateur particulier va favoriser ou défavoriser certains traits floraux. Bien que nos résultats soient restreints à *Impatiens capensis* dans la région de Montréal, ils apportent des éléments nouveaux sur les effets indirects de l'urbanisation sur les plantes. Puisque les communautés de pollinisateurs sont impactées par l'urbanisation, il semblerait que dans un milieu urbain les plantes ayant des fleurs à stratégie de pollinisation généraliste soient plus avantagées que les fleurs spécialistes. Alors qu'une fleur spécialisée pour un type de pollinisateur devrait s'adapter à de nouvelles espèces pollinisatrices, une fleur généraliste sera moins impactée par un changement de pollinisateurs. Nos résultats pourraient ainsi mettre en avant un autre avantage à la stratégie de pollinisation. Nos résultats révèlent également le potentiel d'adaptation d'*Impatiens capensis* face à de nouveaux facteurs environnementaux. Les plantes ne s'adaptent pas toujours facilement dans un nouveau milieu, et des phénomènes de maladaptation ont été observés (Cornille *et al.*, 2018; Lofflin & Kephart, 2005), et ce même pour des espèces généralistes en pollinisation face à de nouvelles communautés de pollinisateurs (Gómez *et al.*, 2009). Ce ne semble cependant pas être le cas pour l'Impatiens du Cap, dont la forme variable permet une bonne adaptation à divers pollinisateurs.

De manière générale les trois études réalisées dans cette thèse ont apporté des éléments nouveaux sur deux points : l'avantage d'être généraliste en pollinisation et l'importance de la forme florale. Avec la deuxième et troisième étude, nous avons démontré que pour les espèces étudiées la stratégie de pollinisation généraliste permettait de s'adapter face à des changements environnementaux. Dans les deux cas, ces changements ont entraîné des modifications dans les communautés de pollinisateurs présents sur les sites d'étude. Grâce à une stratégie de pollinisation généraliste, la pollinisation par plusieurs pollinisateurs différents est possible sur les fleurs des espèces étudiées, ce qui leur permet d'assurer une pollinisation malgré ces changements. Que ce soit en milieu insulaire ou en milieu urbain, la stratégie généraliste semble être optimale pour faire face aux changements de communautés de pollinisateurs. La forme florale est également importante pour assurer une pollinisation dans un contexte de changement de pollinisateur. Nous avons montré avec la première étude que la forme florale, notamment la longueur de la fleur, est corrélée au bec du colibri pour des espèces spécialistes de ce pollinisateur. Ce n'est cependant pas le cas pour des espèces qui sont généralistes. Si un changement dans les communautés de pollinisateurs présents dans le milieu se produit, des fleurs généralistes seront plus à même de s'adapter à la pollinisation par des espèces différentes. C'est ce que nous avons démontré dans les deux autres études réalisées dans cette thèse. La forme florale est importante, autant pour un syndrome de pollinisation spécialiste que généraliste, car elle permet l'attrait de différents pollinisateurs, et permettra le succès de la pollinisation.

5.3. Directions futures

La première étude portait sur les espèces de Gesneriaceae pollinisées uniquement ou en partie (généralistes) par les colibris. Les résultats ont démontré que les fleurs de ces espèces avaient développé une forte adaptation à ce type fonctionnel de pollinisateur, mais aussi aux espèces de colibris qui les pollinisent plus précisément, puisque la longueur des corolles est corrélée à la longueur du bec du pollinisateur chez les espèces spécialistes. Les espèces étudiées étant pollinisées par une ou deux espèces de colibris, la spécialisation de ces fleurs est très précise. Cependant certaines espèces de Gesneriaceae antillaises peuvent aussi être pollinisées par des abeilles, des chauves-souris et parfois même des papillons de nuit. Il serait intéressant de reproduire la méthodologie utilisée dans cette étude pour tester si les fleurs spécialistes de la pollinisation des

chauves-souris par exemple, telle que l'espèce *Gesneria fruticosa*, sont aussi bien adaptées à la forme du museau de ces mammifères.

L'objectif initial du deuxième chapitre de cette thèse, lors de la préparation du terrain et de la mise en place de la méthodologie, était d'étudier la déposition de pollen des différents types de pollinisateur de *Rhytidophyllum bicolor* – colibris, chauves-souris et abeilles – et de les comparer, pour déterminer si ces différents pollinisateurs avaient le même effet sur la valeur sélective de la fleur. En arrivant à Haïti un an après le passage de l'ouragan Matthew, et en ne connaissant pas d'avance l'impact que celui-ci avait eu sur les populations de colibris, nous ne savions pas que ceux-ci seraient absents lors de notre travail sur le terrain. Face à l'absence de pollinisation par les colibris durant notre échantillonnage au Parc du Pic Macaya, nous n'avons pas pu atteindre cet objectif. Cependant plusieurs autres espèces de Gesneriaceae à stratégie généraliste sont présentes sur les îles des Antilles, et certaines dans des zones n'ayant pas été touchées par un ouragan dans les dernières années. Il serait donc important pour une future étude sur cette famille de plante, de réaliser cet objectif.

L'impact considérable de l'ouragan Matthew sur les populations de colibris dans le parc du Pic Macaya apporte une possibilité d'étude originale. Bien qu'il n'y ait pas, à notre connaissance, de rapport antérieur à 2015 qui indique les estimations du nombre de colibris qui étaient présents dans cette zone, il semblerait que la chute du nombre d'oiseaux permettrait de réaliser des études sur plusieurs années de la recolonisation ou le repeuplement de colibris. Quatre espèces de colibris ont été recensés dans le parc, et sur ces quatre espèces, seulement deux individus de l'espèce *Mellisuga minima* et un individu de l'espèce *Archilochus colubris* ont été aperçus lors de nos observations. Le potentiel d'études sur le repeuplement d'au moins une de ces espèces sur le parc du Pic Macaya à travers plusieurs années est intéressant et pourrait être réalisé d'une manière similaire à l'étude de Wiley & Wunderle (1993).

Tel que discuté plus haut, la troisième étude de cette thèse apporte la possibilité de poursuivre l'étude de l'effet de l'urbanisation sur la forme florale sur *Impatiens capensis* mais aussi pour d'autres espèces de plantes. La réalisation d'études similaires sur d'autres espèces de plantes indigènes qui ont également un bon potentiel d'adaptation à la vie urbaine serait utile, puisque

qu'elle permettrait de comparer l'effet de l'urbanisation sur la forme florale pour plusieurs espèces de plantes, et de voir si cet effet est similaire ou différent suivant les espèces. Il serait également intéressant d'étudier d'autres traits floraux. Ici nous avons étudié l'impact de l'urbanisation sur la forme florale qui a été quantifiée par morphométrie. Cependant cette mesure ne prend pas en compte la taille de la fleur, qui pourrait également varier entre les sites et selon les pollinisateurs présents. De précédentes études sur la famille des Balsaminacées ont montré que la quantité et la composition de nectar pouvait également varier en fonction des pollinisateurs (Abrahamczyk *et al.*, 2017; Vandeloock *et al.*, 2019). Il serait donc opportun de mesurer ces traits floraux chez *Impatiens capensis* afin de voir s'ils sont impactés également par les diverses espèces de pollinisateurs visitant cette espèce.

5.4. Conclusion

À travers trois études différentes, nous avons montré que les fleurs sont sujettes à des pressions de sélection très variables. Les pollinisateurs, l'insularité ou l'urbanisation peuvent déclencher des adaptations variées sur la forme florale. Alors que les pollinisateurs vont avoir un effet direct sur l'adaptation florale, par leur comportement de pollinisation ou leur efficacité de pollinisation, l'environnement (climat, urbanisation) va avoir un effet indirect sur les traits floraux. En effet l'environnement agit de manière directe sur la biologie de la plante (temps de floraison, croissance, génétique, etc.) mais a un effet indirect sur la forme florale, puisque que c'est à travers les pollinisateurs que se fait la pression de sélection. Dans ces cas, il semble que la stratégie de pollinisation généraliste soit un atout pour faire face aux variations de pollinisateurs, et la forme florale est cruciale pour assurer un succès de pollinisation. Bien que de nouvelles études sur des espèces différentes ou augmentant l'échantillonnage sont nécessaires, nous avons apporté à travers cette thèse de nouveaux éléments de compréhension de l'impact de l'environnement et des pollinisateurs sur la forme florale.

Références bibliographiques

- Abrahamczyk, S., & Kessler, M. (2010). Hummingbird diversity, food niche characters, and assemblage composition along a latitudinal precipitation gradient in the Bolivian lowlands. *Journal of Ornithology*, 151(3), 615-625.
- Abrahamczyk, S., & Kessler, M. (2015). Morphological and behavioural adaptations to feed on nectar: how feeding ecology determines the diversity and composition of hummingbird assemblages. *Journal of Ornithology*, 156(2), 333-347.
- Abrahamczyk, S., Lozada-Gobilard, S., Ackermann, M., Fischer, E., Krieger, V., Redling, A., & Weigend, M. (2017). A question of data quality—Testing pollination syndromes in Balsaminaceae. *PloS one*, 12(10), e0186125.
- Abrahamczyk, S., Souto-Vilarós, D., McGuire, J. A., & Renner, S. S. (2015). Diversity and clade ages of West Indian hummingbirds and the largest plant clades dependent on them: a 5–9 Myr young mutualistic system. *Biological journal of the Linnean Society*, 114(4), 848-859.
- Adams, D. C., Collyer, M., Kaliontzopoulou, A., & Sherratt, E. (2016). Geomorph: Software for geometric morphometric analyses.
- Agosta, S. J., & Janzen, D. H. (2005). Body size distributions of large Costa Rican dry forest moths and the underlying relationship between plant and pollinator morphology. *Oikos*, 108(1), 183-193.
- Aguilar-Rodríguez, P., Krömer, T., García-Franco, J., & MacSwiney G, M. (2016). From dusk till dawn: nocturnal and diurnal pollination in the epiphyte Tillandsia heterophylla (Bromeliaceae). *Plant Biology*, 18(1), 37-45.
- Aigner, P. (2005). Variation in pollination performance gradients in a Dudleya species complex: can generalization promote floral divergence? *Functional Ecology*, 19(4), 681-689.
- Aigner, P. A. (2001). Optimality modeling and fitness trade-offs: when should plants become pollinator specialists? *Oikos*, 95(1), 177-184.
- Aigner, P. A. (2004). Floral specialization without trade-offs: optimal corolla flare in contrasting pollination environments. *Ecology*, 85(9), 2560-2569.
- Aigner, P. A. (2005). Variation in pollination performance gradients in a Dudleya species complex: can generalization promote floral divergence? *Functional Ecology*, 681-689.
- Aigner, P. A. (2006). The evolution of specialized floral phenotypes in a fine-grained pollination environment. *Plant-pollinator interactions: From specialization to generalization*, 23-46.
- Alberti, M., Correa, C., Marzluff, J. M., Hendry, A. P., Palkovacs, E. P., Gotanda, K. M., Hunt, V. M., Apgar, T. M., & Zhou, Y. (2017). Global urban signatures of phenotypic change in animal and plant populations. *Proceedings of the National Academy of Sciences*, 114(34), 8951-8956.
- Alexandre, H., Vrignaud, J., Mangin, B., & Joly, S. (2015). Genetic architecture of pollination syndrome transition between hummingbird-specialist and generalist species in the genus Rhytidophyllum (Gesneriaceae). *PeerJ*, 3, e1028.

- Altermatt, F., & Ebert, D. (2016). Reduced flight-to-light behaviour of moth populations exposed to long-term urban light pollution. *Biology letters*, 12(4), 20160111.
- Anderson, B., & Johnson, S. D. (2009). Geographical covariation and local convergence of flower depth in a guild of fly-pollinated plants. *New Phytologist*, 182(2), 533-540.
- Araujo, A. C., & Sazima, M. (2003). The assemblage of flowers visited by hummingbirds in the “capões” of Southern Pantanal, Mato Grosso do Sul, Brazil. *Flora-Morphology, Distribution, Functional Ecology of Plants*, 198(6), 427-435.
- Armbruster, S., Fenster, C., & Dudash, M. (2000). Pollination 'principles' revisited: specialization, pollination syndromes, and the evolution of flowers. *The Scandinavian Association for Pollination Ecology Honours Knut Faegri*, 39, 179-200.
- Armbruster, W. S. (2014). Floral specialization and angiosperm diversity: phenotypic divergence, fitness trade-offs and realized pollination accuracy. *AoB Plants*, 6. doi:10.1093/aobpla/plu003
- Armbruster, W. S., & Baldwin, B. G. (1998). Switch from specialized to generalized pollination. *Nature*, 394(6694), 632.
- Armbruster, W. S., Pélabon, C., Bolstad, G. H., & Hansen, T. F. (2014). Integrated phenotypes: understanding trait covariation in plants and animals. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 369(1649), 20130245.
- Ashman, T.-L., & Stanton, M. (1991). Seasonal variation in pollination dynamics of sexually dimorphic *Sidalcea oregana* ssp. *spicata* (Malvaceae). *Ecology*, 72(3), 993-1003.
- Baker, H. (1961). The adaptation of flowering plants to nocturnal and crepuscular pollinators. *The Quarterly Review of Biology*, 36(1), 64-73.
- Baker, H. G., & Baker, I. (1990). The predictive value of nectar chemistry to the recognition of pollinator types. *Israel Journal of Plant Sciences*, 39(1-2), 157-166.
- Barker, C. (2018). *Impacts of an urbanization gradient on pollination services to a bee-pollinated plant*. Université d'Ottawa/University of Ottawa,
- Barker, C. A., & Sargent, R. D. (2020). Pollination Services to *Impatiens Capensis* (Balsaminaceae) Are Maintained across an Urbanization Gradient. *International Journal of Plant Sciences*, 181(9), 000-000.
- Barrett, S. C., Emerson, B., & Mallet, J. (1996). The reproductive biology and genetics of island plants [and discussion]. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 351(1341), 725-733.
- Bates, A. J., Sadler, J. P., Fairbrass, A. J., Falk, S. J., Hale, J. D., & Matthews, T. J. (2011). Changing bee and hoverfly pollinator assemblages along an urban-rural gradient. *PloS one*, 6(8), e23459.
- Beaufort-Murphy, H. T. (1983). The seed surface morphology of the Gesneriaceae utilizing the scanning electron microscope and a new system for diagnosing seed morphology. *Selbyana*(6), 220-422.

- Berns, C. M., & Adams, D. C. (2010). Bill shape and sexual shape dimorphism between two species of temperate hummingbirds: Black-Chinned hummingbird (*Archilochus alexandri*) and Ruby-Throated hummingbird (*A. colubris*). *The Auk*, 127(3), 626-635.
- Berry, J., & Bjorkman, O. (1980). Photosynthetic response and adaptation to temperature in higher plants. *Annual Review of plant physiology*, 31(1), 491-543.
- Brosi, B. J. (2016). Pollinator specialization: from the individual to the community. *New Phytologist*, 210(4), 1190-1194.
- Burdine, J. D., & McCluney, K. E. (2019). Interactive effects of urbanization and local habitat characteristics influence bee communities and flower visitation rates. *Oecologia*, 190(4), 715-723.
- Cam, E., Nichols, J. D., Sauer, J. R., Hines, J. E., & Flather, C. H. (2000). Relative species richness and community completeness: birds and urbanization in the Mid-Atlantic States. *Ecological Applications*, 10(4), 1196-1210.
- Cárdenas, S., Niveló-Villavicencio, C., Cárdenas, J. D., Landázuri, O., & Tinoco, B. A. (2017). First record of flower visitation by a rodent in Neotropical Proteaceae, *Oreocallis grandiflora*. *Journal of Tropical Ecology*, 33(2), 174-177.
- Carper, A. L., Adler, L. S., Warren, P. S., & Irwin, R. E. (2014). Effects of suburbanization on forest bee communities. *Environmental entomology*, 43(2), 253-262.
- Castellanos, M., Wilson, P., & Thomson, J. (2004). 'Anti-bee' and 'pro-bird' changes during the evolution of hummingbird pollination in *Penstemon* flowers. *Journal of evolutionary biology*, 17(4), 876-885.
- Castellanos, M. C., Wilson, P., & Thomson, J. D. (2003). Pollen transfer by hummingbirds and bumblebees, and the divergence of pollination modes in *Penstemon*. *Evolution*, 57(12), 2742-2752.
- Chase, M. W., & Hills, H. G. (1992). Orchid phylogeny, flower sexuality, and fragrance-seeking. *BioScience*, 42(1), 43-49.
- Chew, F., & Courtney, S. (1991). Plant apparency and evolutionary escape from insect herbivory. *The American Naturalist*, 138(3), 729-750.
- Clark J.L., L.E. Skog, J.K. Boggan, & Ginzburg, a. S. (2020). Index to names of New World members of the Gesneriaceae (subfamilies Sanangoideae and Gesnerioideae). *Rhedeia* 30(1): 190-256.
- Coleman, J. L., & Barclay, R. M. (2011). Influence of urbanization on demography of little brown bats (*Myotis lucifugus*) in the prairies of North America. *PloS one*, 6(5), e20483.
- Colles, A., Liow, L. H., & Prinzing, A. (2009). Are specialists at risk under environmental change? Neoecological, paleoecological and phylogenetic approaches. *Ecology Letters*, 12(8), 849-863.
- Cornille, A., Salcedo, A., Huang, H., Kryvokhyzha, D., Holm, K., Ge, X., Stinchcombe, J., Glemin, S., Wright, S., & Lascoux, M. (2018). Local adaptation and maladaptation during the worldwide range expansion of a selffertilizing plant. *bioRxiv*, 308619.
- Cotton, P. A. (1998). Coevolution in an Amazonian hummingbird-plant community. *Ibis*, 140(4), 639-646.

- Cresswell, J. (1998). Stabilizing selection and the structural variability of flowers within species. *Annals of Botany*, 81(4), 463-473.
- Croci, S., Butet, A., & Clergeau, P. (2008). Does urbanization filter birds on the basis of their biological traits. *The Condor*, 110(2), 223-240.
- Dalsgaard, B., González, A. M. M., Olesen, J. M., Ollerton, J., Timmermann, A., Andersen, L. H., & Tossas, A. G. (2009). Plant-hummingbird interactions in the West Indies: floral specialisation gradients associated with environment and hummingbird size. *Oecologia*, 159(4), 757-766.
- Dalsgaard, B., Martín González, A. M., Olesen, J. M., Timmermann, A., Andersen, L. H., & Ollerton, J. (2008). Pollination networks and functional specialization: a test using Lesser Antillean plant-hummingbird assemblages. *Oikos*, 117(5), 789-793.
- Darwin, C. (1862). *On the Various Contrivances by Which British and Foreign Orchids are Fertilised by Insects: And on the Good Effect of Intercrossing*: Cambridge Library Collection.
- Darwin, C. (1876). The effects of cross and self fertilization in the vegetable kingdom. 1876 London. UK: John Murray.
- de Brito, V. L., Rech, A. R., Ollerton, J., & Sazima, M. (2017). Nectar production, reproductive success and the evolution of generalised pollination within a specialised pollen-rewarding plant family: a case study using *Miconia theizans*. *Plant Systematics and Evolution*, 1-10.
- de Castro, E. C., Zagrobelny, M., Cardoso, M. Z., & Bak, S. (2018). The arms race between heliconiine butterflies and *Passiflora* plants—new insights on an ancient subject. *Biological Reviews*, 93(1), 555-573.
- del Coro Arizmendi, M., & Ornelas, J. F. (1990). Hummingbirds and their floral resources in a tropical dry forest in Mexico. *Biotropica*, 172-180.
- Dellinger, A. S. (2020). Pollination syndromes in the 21st century: where do we stand and where may we go? *New Phytologist*.
- Dellinger, A. S., Chartier, M., Fernández-Fernández, D., Penneys, D. S., Alvear, M., Almeda, F., Michelangeli, F. A., Staedler, Y., Armbruster, W. S., & Schönenberger, J. (2019). Beyond buzz-pollination—departures from an adaptive plateau lead to new pollination syndromes. *New Phytologist*, 221(2), 1136-1149.
- Dohzono, I., Takami, Y., & Suzuki, K. (2011). Is bumblebee foraging efficiency mediated by morphological correspondence to flowers? *International Journal of Insect Science*, 3, IJIS. S4758.
- Dolan, R. W., Moore, M. E., & Stephens, J. D. (2011). Documenting effects of urbanization on flora using herbarium records. *Journal of Ecology*, 99(4), 1055-1062.
- Donihue, C. M., Herrel, A., Fabre, A.-C., Kamath, A., Geneva, A. J., Schoener, T. W., Kolbe, J. J., & Losos, J. B. (2018). Hurricane-induced selection on the morphology of an island lizard. *Nature*, 560(7716), 88.
- Drusch, M., Del Bello, U., Carlier, S., Colin, O., Fernandez, V., Gascon, F., Hoersch, B., Isola, C., Laberinti, P., & Martimort, P. (2012). Sentinel-2: ESA's optical high-resolution mission for GMES operational services. *Remote sensing of Environment*, 120, 25-36.

- Esau, I., Miles, V. V., Davy, R., Miles, M. W., & Kurchatova, A. (2016). Trends in normalized difference vegetation index (NDVI) associated with urban development in northern West Siberia. *Atmospheric Chemistry & Physics*, 16(15).
- Etcheverry, Á. V., Figueroa-Castro, D., Figueroa-Fleming, T., Alemán, M. M., Juárez, V. D., López-Spahr, D., Yáñez, C. N., & Gómez, C. A. (2012). Generalised pollination system of *Erythrina dominguezii* (Fabaceae: Papilionoideae) involving hummingbirds, passerines and bees. *Australian Journal of Botany*, 60(6), 484-494.
- Faegri, K. L. van der Pijl. 1966. The principles of pollination ecology. *Pergamon Press, New York. Faegri The principles of pollination ecology* 1966.
- Faegri, K., & Van Der Pijl, L. (2013). *Principles of pollination ecology*: Elsevier.
- Feinsinger, P., Murray, K. G., Kinsman, S., & Busby, W. H. (1986). Floral neighborhood and pollination success in four hummingbird-pollinated cloud forest plant species. *Ecology*, 67(2), 449-464.
- Fenster, C. B., Armbruster, W. S., Wilson, P., Dudash, M. R., & Thomson, J. D. (2004). Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution, and Systematics*, 375-403.
- Fisogni, A., Hautekèete, N., Piquot, Y., Brun, M., Vanappelghem, C., Michez, D., & Massol, F. (2020). Urbanization drives an early spring for plants but not for pollinators. *Oikos*.
- Foster, D., Podos, J., & Hendry, A. (2008). A geometric morphometric appraisal of beak shape in Darwin's finches. *Journal of evolutionary biology*, 21(1), 263-275.
- Freitas, L. (2013). Concepts of pollinator performance: is a simple approach necessary to achieve a standardized terminology? *Brazilian Journal of Botany*, 36(1), 3-8.
- Fumero-Cabán, J. J., & Meléndez-Ackerman, E. J. (2007). Relative pollination effectiveness of floral visitors of *Pitcairnia angustifolia* (Bromeliaceae). *American Journal of Botany*, 94(3), 419-424.
- Galen, C. (1989). Measuring pollinator-mediated selection on morphometric floral traits: bumblebees and the alpine sky pilot, *Polemonium viscosum*. *Evolution*, 43(4), 882-890.
- Geslin, B., Gauzens, B., Thebault, E., & Dajoz, I. (2013). Plant pollinator networks along a gradient of urbanisation. *PloS one*, 8(5), e63421.
- Gómez, J. M., Abdelaziz, M., Camacho, J. P. M., Muñoz-Pajares, A. J., & Perfectti, F. (2009). Local adaptation and maladaptation to pollinators in a generalist geographic mosaic. *Ecology Letters*, 12(7), 672-682.
- Gómez, J. M., Perfectti, F., & Klingenberg, C. P. (2014). The role of pollinator diversity in the evolution of corolla-shape integration in a pollination-generalist plant clade. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 369(1649), 20130257.
- Gómez, J. M., Perfectti, F., & Lorite, J. (2015). The role of pollinators in floral diversification in a clade of generalist flowers. *Evolution*, 69(4), 863-878.
- Gómez, J. M., Torices, R., Lorite, J., Klingenberg, C. P., & Perfectti, F. (2016). The role of pollinators in the evolution of corolla shape variation, disparity and integration in a highly diversified plant family with a conserved floral bauplan. *Annals of Botany*, 117(5), 889-904.

- Gómez, J. M., & Zamora, R. (2006). Ecological factors that promote the evolution of generalization in pollination systems. *Plant-pollinator interactions*, NM Waser, J. Ollerton (eds.). The University of Chicago Press, Chicago, 145-166.
- González-Varo, J. P., Biesmeijer, J. C., Bommarco, R., Potts, S. G., Schweiger, O., Smith, H. G., Steffan-Dewenter, I., Szentgyörgyi, H., Woyciechowski, M., & Vilà, M. (2013). Combined effects of global change pressures on animal-mediated pollination. *Trends in Ecology & Evolution*, 28(9), 524-530.
- Gortat, T., Barkowska, M., Tkowska, A. G.-S., Pieniążek, A., Kozakiewicz, A., & Kozakiewicz, M. (2014). The effects of urbanization—small mammal communities in a gradient of human pressure in Warsaw city, Poland. *Polish Journal of Ecology*, 62(1), 163-172.
- Grant, V., & Grant, K. A. (1965). Flower pollination in the Phlox family.
- Gregg, J. W., Jones, C. G., & Dawson, T. E. (2003). Urbanization effects on tree growth in the vicinity of New York City. *Nature*, 424(6945), 183-187.
- Hall, D. M., Camilo, G. R., Tonietto, R. K., Ollerton, J., Ahrné, K., Arduser, M., Ascher, J. S., Baldock, K. C., Fowler, R., & Frankie, G. (2017). The city as a refuge for insect pollinators. *Conservation Biology*, 31(1), 24-29.
- Hamblin, A. L., Youngsteadt, E., & Frank, S. D. (2018). Wild bee abundance declines with urban warming, regardless of floral density. *Urban ecosystems*, 21(3), 419-428.
- Hedges, S. B., Cohen, W. B., Timyan, J., & Yang, Z. (2018). Haiti's biodiversity threatened by nearly complete loss of primary forest. *Proceedings of the National Academy of Sciences*, 115(46), 11850-11855.
- Herrera, C. M. (1996). Floral traits and plant adaptation to insect pollinators: a devil's advocate approach. In *Floral biology* (pp. 65-87): Springer.
- Herrera, C. M. (2005). Plant generalization on pollinators: species property or local phenomenon? *American Journal of Botany*, 92(1), 13-20.
- Hsu, H.-C., Chou, W.-C., & Kuo, Y.-F. (2020). 3D revelation of phenotypic variation, evolutionary allometry, and ancestral states of corolla shape: a case study of clade *Corytholoma* (subtribe Ligeriinae, family Gesneriaceae). *GigaScience*, 9(1), giz155.
- Hung, K.-L. J., Ascher, J. S., & Holway, D. A. (2017). Urbanization-induced habitat fragmentation erodes multiple components of temporal diversity in a Southern California native bee assemblage. *PloS one*, 12(8), e0184136.
- Johnson, M. T., & Munshi-South, J. (2017). Evolution of life in urban environments. *Science*, 358(6363), eaam8327.
- Johnson, M. T., Thompson, K. A., & Saini, H. S. (2015). Plant evolution in the urban jungle. *American Journal of Botany*, 102(12), 1951-1953.
- Johnson, S. D., & Steiner, K. E. (2000). Generalization versus specialization in plant pollination systems. *Trends in Ecology & Evolution*, 15(4), 140-143.
- Joly, S., Lambert, F., Alexandre, H., Clavel, J., Léveillé-Bourret, É., & Clark, J. L. (2018). Greater pollination generalization is not associated with reduced constraints on corolla shape in Antillean plants. *Evolution*, 72(2), 244-260.
- Jump, A. S., & Peñuelas, J. (2005). Running to stand still: adaptation and the response of plants to rapid climate change. *Ecology Letters*, 8(9), 1010-1020.

- Kaczorowski, R. L., Seliger, A. R., Gaskett, A. C., Wigsten, S. K., & Raguso, R. A. (2012). Corolla shape vs. size in flower choice by a nocturnal hawkmoth pollinator. *Functional Ecology*, 26(3), 577-587.
- Kessler, A., & Baldwin, I. T. (2002). Plant responses to insect herbivory: the emerging molecular analysis. *Annual review of plant biology*, 53(1), 299-328.
- King, S. A., & Buckney, R. T. (2000). Urbanization and exotic plants in northern Sydney streams. *Austral Ecology*, 25(5), 455-461.
- Kodric-Brown, A., Brown, J. H., Byers, G. S., & Gori, D. F. (1984). Organization of a tropical island community of hummingbirds and flowers. *Ecology*, 65(5), 1358-1368.
- Lagomarsino, L. P., Forrestel, E. J., Muchhala, N., & Davis, C. C. (2017). Repeated evolution of vertebrate pollination syndromes in a recently diverged Andean plant clade. *Evolution*, 71(8), 1970-1985.
- Lagomarsino, L. P., & Muchhala, N. (2019). A gradient of pollination specialization in three species of Bolivian Centropogon. *American Journal of Botany*.
- Larson, J. L., Kesheimer, A. J., & Potter, D. A. (2014). Pollinator assemblages on dandelions and white clover in urban and suburban lawns. *Journal of insect conservation*, 18(5), 863-873.
- Law, B. S., & Lean, M. (1999). Common blossom bats (*Syconycteris australis*) as pollinators in fragmented Australian tropical rainforest. *Biological Conservation*, 91(2-3), 201-212.
- Lechowicz, M. J., Schoen, D. J., & Bell, G. (1988). Environmental correlates of habitat distribution and fitness components in *Impatiens capensis* and *Impatiens pallida*. *The Journal of Ecology*, 1043-1054.
- Legendre, P., & Gallagher, E. D. (2001). Ecologically meaningful transformations for ordination of species data. *Oecologia*, 129(2), 271-280.
- Leong, M., Ponisio, L. C., Kremen, C., Thorp, R. W., & Roderick, G. K. (2016). Temporal dynamics influenced by global change: bee community phenology in urban, agricultural, and natural landscapes. *Global change biology*, 22(3), 1046-1053.
- Lofflin, D. L., & Kephart, S. R. (2005). Outbreeding, seedling establishment, and maladaptation in natural and reintroduced populations of rare and common *Silene douglasii* (Caryophyllaceae). *American Journal of Botany*, 92(10), 1691-1700.
- Łopucki, R., Mróz, I., Berliński, Ł., & Burzych, M. (2013). Effects of urbanization on small-mammal communities and the population structure of synurbic species: an example of a medium-sized city. *Canadian journal of zoology*, 91(8), 554-561.
- Lu, Y. (2002). Why is cleistogamy a selected reproductive strategy in *Impatiens capensis* (Balsaminaceae)? *Biological journal of the Linnean Society*, 75(4), 543-553.
- Lunau, K. (2004). Adaptive radiation and coevolution—pollination biology case studies. *Organisms Diversity & Evolution*, 4(3), 207-224.
- Maglianesi, M. A., Blüthgen, N., Böhning-Gaese, K., & Schleuning, M. (2014). Morphological traits determine specialization and resource use in plant-hummingbird networks in the neotropics. *Ecology*, 95(12), 3325-3334.

- Maglianesi, M. A., Böhning-Gaese, K., & Schleuning, M. (2015). Different foraging preferences of hummingbirds on artificial and natural flowers reveal mechanisms structuring plant-pollinator interactions. *Journal of Animal Ecology*, 84(3), 655-664.
- Marschner, H. (1991). Mechanisms of adaptation of plants to acid soils. *Plant and soil*, 134(1), 1-20.
- Martén-Rodríguez, S., & Fenster, C. B. (2008). Pollination ecology and breeding systems of five Gesneria species from Puerto Rico. *Annals of Botany*, 102(1), 23-30.
- Martén-Rodríguez, S., Almarales-Castro, A., & Fenster, C. B. (2009). Evaluation of pollination syndromes in Antillean Gesneriaceae: evidence for bat, hummingbird and generalized flowers. *Journal of Ecology*, 97(2), 348-359.
- Martén-Rodríguez, S., Fenster, C. B., Agnarsson, I., Skog, L. E., & Zimmer, E. A. (2010). Evolutionary breakdown of pollination specialization in a Caribbean plant radiation. *New Phytologist*, 188(2), 403-417.
- Martén-Rodríguez, S., Quesada, M., Castro, A. A., Lopezaraiza-Mikel, M., & Fenster, C. B. (2015). A comparison of reproductive strategies between island and mainland Caribbean Gesneriaceae. *Journal of Ecology*, 103(5), 1190-1204.
- Martins, K. T., Gonzalez, A., & Lechowicz, M. J. (2017). Patterns of pollinator turnover and increasing diversity associated with urban habitats. *Urban ecosystems*, 20(6), 1359-1371.
- Marzluff, J. M. (2001). Worldwide urbanization and its effects on birds. In *Avian ecology and conservation in an urbanizing world* (pp. 19-47): Springer.
- Matteson, K., Grace, J. B., & Minor, E. (2013). Direct and indirect effects of land use on floral resources and flower-visiting insects across an urban landscape. *Oikos*, 122(5), 682-694.
- Mayfield, M. M., Waser, N. M., & Price, M. V. (2001). Exploring the 'most effective pollinator principle' with complex flowers: bumblebees and *Ipomopsis aggregata*. *Annals of Botany*, 88(4), 591-596.
- McFrederick, Q. S., & LeBuhn, G. (2006). Are urban parks refuges for bumble bees *Bombus* spp. (Hymenoptera: Apidae)? *Biological Conservation*, 129(3), 372-382.
- McKinney, M. L. (1997). Extinction vulnerability and selectivity: combining ecological and paleontological views. *Annual Review of Ecology and Systematics*, 28(1), 495-516.
- McKinney, M. L. (2002). Urbanization, Biodiversity, and Conservation The impacts of urbanization on native species are poorly studied, but educating a highly urbanized human population about these impacts can greatly improve species conservation in all ecosystems. *Bioscience*, 52(10), 883-890.
- McKinney, M. L. (2008). Effects of urbanization on species richness: a review of plants and animals. *Urban ecosystems*, 11(2), 161-176.
- Merckx, T., Kaiser, A., & Van Dyck, H. (2018). Increased body size along urbanization gradients at both community and intraspecific level in macro-moths. *Global change biology*, 24(8), 3837-3848.

- Muchhala, N. (2003). Exploring the boundary between pollination syndromes: bats and hummingbirds as pollinators of *Burmeistera cyclostigmata* and *B. tenuiflora* (Campanulaceae). *Oecologia*, 134(3), 373-380.
- Muchhala, N. (2006). The pollination biology of *Burmeistera* (Campanulaceae): specialization and syndromes. *American Journal of Botany*, 93(8), 1081-1089.
- Muchhala, N. (2007a). Adaptive trade-off in floral morphology mediates specialization for flowers pollinated by bats and hummingbirds. *The American Naturalist*, 169(4), 494-504.
- Muchhala, N. (2007b). Adaptive trade-off in floral morphology mediates specialization for flowers pollinated by bats and hummingbirds. *The American Naturalist*, 169(4), 494-504.
- Muchhala, N., & Potts, M. D. (2007). Character displacement among bat-pollinated flowers of the genus *Burmeistera*: analysis of mechanism, process and pattern. *Proceedings of the Royal Society of London B: Biological Sciences*, 274(1626), 2731-2737.
- Muchhala, N., & Thomson, J. D. (2010). Fur versus feathers: pollen delivery by bats and hummingbirds and consequences for pollen production. *The American Naturalist*, 175(6), 717-726.
- Nassar, J. M., Ramírez, N., & Linares, O. (1997). Comparative pollination biology of Venezuelan columnar cacti and the role of nectar-feeding bats in their sexual reproduction. *American Journal of Botany*, 84(7), 918-927.
- Nattero, J., & Cocucci, A. A. (2007). Geographical variation in floral traits of the tree tobacco in relation to its hummingbird pollinator fauna. *Biological journal of the Linnean Society*, 90(4), 657-667.
- Ne'eman, G., Jürgens, A., Newstrom-Lloyd, L., Potts, S. G., & Dafni, A. (2010). A framework for comparing pollinator performance: effectiveness and efficiency. *Biological Reviews*, 85(3), 435-451.
- Neil, K., & Wu, J. (2006). Effects of urbanization on plant flowering phenology: a review. *Urban ecosystems*, 9(3), 243-257.
- Neil, K., Wu, J., Bang, C., & Faeth, S. (2014). Urbanization affects plant flowering phenology and pollinator community: effects of water availability and land cover. *Ecological Processes*, 3(1), 17.
- Niemirski, R., & Zych, M. (2011). Fly pollination of dichogamous *Angelica sylvestris* (Apiaceae): how (functionally) specialized can a (morphologically) generalized plant be? *Plant Systematics and Evolution*, 294(3-4), 147-158.
- Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'hara, R., Simpson, G. L., Solymos, P., Stevens, M. H. H., & Wagner, H. (2013). Package 'vegan'. *Community ecology package, version*, 2(9), 1-295.
- Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Stevens, M. H. H., Oksanen, M. J., & Suggests, M. (2007). The vegan package. *Community ecology package*, 10, 631-637.
- Olesen, J. M. (2000). Exactly how generalised are pollination interactions. *Det Norske Videnskaps-Akademi. I. Matematisk Naturvidenssskapelige Klasse, Skrifter, Ny Serie*, 39, 161-178.

- Olesen, J. M., Eskildsen, L. I., & Venkatasamy, S. (2002). Invasion of pollination networks on oceanic islands: importance of invader complexes and endemic super generalists. *Diversity and Distributions*, 8(3), 181-192.
- Olesen, J. M., & Jordano, P. (2002). Geographic patterns in plant–pollinator mutualistic networks. *Ecology*, 83(9), 2416-2424.
- Ollerton, J., Alarcón, R., Waser, N. M., Price, M. V., Watts, S., Cranmer, L., Hingston, A., Peter, C. I., & Rotenberry, J. (2009). A global test of the pollination syndrome hypothesis. *Annals of botany*, 103(9), 1471-1480.
- Ollerton, J., & Cranmer, L. (2002). Latitudinal trends in plant-pollinator interactions: are tropical plants more specialised? *Oikos*, 98(2), 340-350.
- Ollerton, J., Johnson, S. D., & Hingston, A. B. (2006). Geographical Variation in Diversity and Specificity of Pollination Systems. *Plant-pollinator interactions: from specialization to generalization*, 283.
- Ollerton, J., Killick, A., Lamborn, E., Watts, S., & Whiston, M. (2007). Multiple meanings and modes: on the many ways to be a generalist flower. *Taxon*, 56(3), 717-728.
- Ollerton, J., Winfree, R., & Tarrant, S. (2011). How many flowering plants are pollinated by animals? *Oikos*, 120(3), 321-326.
- Olsen, K. M. (1996). Pollination effectiveness and pollinator importance in a population of *Heterotheca subaxillaris* (Asteraceae). *Oecologia*, 109(1), 114-121.
- Ordeñana, M. A., Crooks, K. R., Boydston, E. E., Fisher, R. N., Lyren, L. M., Siudyla, S., Haas, C. D., Harris, S., Hathaway, S. A., & Turschak, G. M. (2010). Effects of urbanization on carnivore species distribution and richness. *Journal of Mammalogy*, 91(6), 1322-1331.
- Park, M. G., Raguso, R. A., Losey, J. E., & Danforth, B. N. (2016). Per-visit pollinator performance and regional importance of wild *Bombus* and *Andrena* (*Melandrena*) compared to the managed honey bee in New York apple orchards. *Apidologie*, 47(2), 145-160.
- Peguero, B., Clase, T., & Hilaire, J. (2006). Plants observed during the scientific expedition to Massif de La Hotte, Haiti. *Macaya biodiversité. Expédition scientifique dans la Réserve de la Biosphère de Macaya/2006.*, 58-73.
- Pellmyr, O. (2002). Pollination by animals. *Plant–animal interactions, an evolutionary approach*, 157-184.
- Perret, M., Chautems, A., Spichiger, R., Kite, G., & Savolainen, V. (2003). Systematics and evolution of tribe Sinningieae (Gesneriaceae): evidence from phylogenetic analyses of six plastid DNA regions and nuclear ncpGS. *American Journal of Botany*, 90(3), 445-460.
- Perret, M., Chautems, A., Spichiger, R., Peixoto, M., & Savolainen, V. (2000). Nectar sugar composition in relation to pollination syndromes in Sinningieae (Gesneriaceae). *Annals of Botany*, 87(2), 267-273.
- Pettorelli, N., Ryan, S., Mueller, T., Bunnefeld, N., Jędrzejewska, B., Lima, M., & Kausrud, K. (2011). The Normalized Difference Vegetation Index (NDVI): unforeseen successes in animal ecology. *Climate research*, 46(1), 15-27.
- Plascencia, M., & Philpott, S. (2017). Floral abundance, richness, and spatial distribution drive urban garden bee communities. *Bulletin of entomological research*, 107(5), 658-667.

- Poisot, T., Canard, E., Mouquet, N., & Hochberg, M. E. (2012). A comparative study of ecological specialization estimators. *Methods in Ecology and Evolution*, 3(3), 537-544.
- Potter, A., & LeBuhn, G. (2015). Pollination service to urban agriculture in San Francisco, CA. *Urban ecosystems*, 18(3), 885-893.
- Prange, S., & Gehrt, S. D. (2004). Changes in mesopredator-community structure in response to urbanization. *Canadian journal of zoology*, 82(11), 1804-1817.
- Queiroz, J., Quirino, Z., Lopes, A., & Machado, I. (2016). Vertebrate mixed pollination system in *Encholirium spectabile*: a bromeliad pollinated by bats, opossum and hummingbirds in a tropical dry forest. *Journal of Arid Environments*, 125, 21-30.
- Quistberg, R. D., Bichier, P., & Philpott, S. M. (2016). Landscape and local correlates of bee abundance and species richness in urban gardens. *Environmental entomology*, 45(3), 592-601.
- Raia, P., Carotenuto, F., Mondanaro, A., Castiglione, S., Passaro, F., Saggese, F., Melchionna, M., Serio, C., Alessio, L., & Silvestro, D. (2016). Progress to extinction: increased specialisation causes the demise of animal clades. *Scientific reports*, 6(1), 1-10.
- Rasband, W. S. (1997). ImageJ. In: Bethesda, MD.
- Rathcke, B. J. (2000). Hurricane Causes Resource And Pollination Limitation Of Fruit Set In A Bird-Pollinated Shrub. *Ecology*, 81(7), 1951-1958.
- Rivkin, L. R., Nhan, V. J., Weis, A. E., & Johnson, M. T. (2020). Variation in pollinator-mediated plant reproduction across an urbanization gradient. *Oecologia*, 1-11.
- Rivkin, L. R., Santangelo, J. S., Alberti, M., Aronson, M. F., de Keyser, C. W., Diamond, S. E., Fortin, M. J., Frazee, L. J., Gorton, A. J., & Hendry, A. P. (2019). A roadmap for urban evolutionary ecology. *Evolutionary Applications*, 12(3), 384-398.
- Roalson, E. H., & Roberts, W. R. (2016). Distinct processes drive diversification in different clades of Gesneriaceae. *Systematic biology*, 65(4), 662-684.
- Rogers, S. R., Tarpy, D. R., & Burrack, H. J. (2013). Multiple criteria for evaluating pollinator performance in highbush blueberry (Ericales: Ericaceae) agroecosystems. *Environmental entomology*, 42(6), 1201-1209.
- Rohlf, F. (2004). TpsDig. *Department of Ecology and Evolution, State University of New York, Stony Brook, NY*.
- Rosas-Guerrero, V., Aguilar, R., Martén-Rodríguez, S., Ashworth, L., Lopezaraiza-Mikel, M., Bastida, J. M., & Quesada, M. (2014). A quantitative review of pollination syndromes: do floral traits predict effective pollinators? *Ecology Letters*, 17(3), 388-400.
- Russo, D., & Ancillotto, L. (2015). Sensitivity of bats to urbanization: a review. *Mammalian Biology*, 80(3), 205-212.
- Rust, R. W. (1977). Pollination in *Impatiens capensis* and *Impatiens pallida* (Balsaminaceae). *Bulletin of the Torrey Botanical Club*, 361-367.
- Sahli, H. F., & Conner, J. K. (2007). Visitation, effectiveness, and efficiency of 15 genera of visitors to wild radish, *Raphanus raphanistrum* (Brassicaceae). *American Journal of Botany*, 94(2), 203-209.

- Sahli, H. F., & Conner, J. K. (2011). Testing for conflicting and nonadditive selection: floral adaptation to multiple pollinators through male and female fitness. *Evolution: International Journal of Organic Evolution*, 65(5), 1457-1473.
- Samuelson, A. E., Gill, R. J., Brown, M. J., & Leadbeater, E. (2018). Lower bumblebee colony reproductive success in agricultural compared with urban environments. *Proceedings of the Royal Society B: Biological Sciences*, 285(1881), 20180807.
- Sanmartin-Gajardo, I., & Sazima, M. (2005). Chiropterophily in Sinningieae (Gesneriaceae): Sinningia brasiliensis and Paliavana prasinata are bat-pollinated, but P. sericiflora is not. Not yet? *Annals of Botany*, 95(7), 1097-1103.
- Santangelo, J. S., Rivkin, L. R., & Johnson, M. T. (2018). The evolution of city life. In: The Royal Society.
- Schmidt, C., Domaratzki, M., Kinnunen, R., Bowman, J., & Garroway, C. J. (2020). Continent-wide effects of urbanization on bird and mammal genetic diversity. *Proceedings of the Royal Society B*, 287(1920), 20192497.
- Shahabi, H., Ahmad, B. B., Mokhtari, M. H., & Zadeh, M. A. (2012). Detection of urban irregular development and green space destruction using normalized difference vegetation index (NDVI), principal component analysis (PCA) and post classification methods: A case study of Saqqez city. *International Journal of Physical Sciences*, 7(17), 2587-2595.
- Shannon, M. C. (1997). Adaptation of plants to salinity. In *Advances in agronomy* (Vol. 60, pp. 75-120): Elsevier.
- Skog, L. E. (1976). A study of the tribe Gesnerieae, with a revision of Gesneria (Gesneriaceae: Gesnerioideae). *Smithsonian Contrib. Bot*, 29, 1-182.
- Smith, S. D., & Kriebel, R. (2018). Convergent evolution of floral shape tied to pollinator shifts in Iochrominae (Solanaceae). *Evolution*, 72(3), 688-697.
- Snow, D. W., & Snow, D. (1980). Relationships between hummingbirds and flowers in the Andes of Colombia.
- Soga, M., Yamaura, Y., Koike, S., & Gaston, K. J. (2014). Land sharing vs. land sparing: does the compact city reconcile urban development and biodiversity conservation? *Journal of Applied ecology*, 51(5), 1378-1386.
- Somme, L., Moquet, L., Quinet, M., Vanderplanck, M., Michez, D., Lognay, G., & Jacquemart, A.-L. (2016). Food in a row: urban trees offer valuable floral resources to pollinating insects. *Urban ecosystems*, 19(3), 1149-1161.
- Sonne, J., Vizentin-Bugoni, J., Maruyama, P. K., Araujo, A. C., Chávez-González, E., Coelho, A. G., Cotton, P. A., Marín-Gómez, O. H., Lara, C., & Lasprilla, L. R. (2020). Ecological mechanisms explaining interactions within plant-hummingbird networks: morphological matching increases towards lower latitudes. *Proceedings of the Royal Society B*, 287(1922), 20192873.
- Spiller, D. A., Losos, J. B., & Schoener, T. W. (1998). Impact of a catastrophic hurricane on island populations. *Science*, 281(5377), 695-697.
- Stark, J. R., Aiello-Lammens, M., & Grigione, M. M. (2020). The effects of urbanization on carnivores in the New York metropolitan area. *Urban ecosystems*, 23(2), 215-225.

- Stebbins, G. L. (1970). Adaptive radiation of reproductive characteristics in angiosperms, I: pollination mechanisms. *Annual Review of Ecology and Systematics*, 307-326.
- Stiles, F. G. (1975). Ecology, flowering phenology, and hummingbird pollination of some Costa Rican *Heliconia* species. *Ecology*, 56(2), 285-301.
- Temeles, E. J. (1996). A new dimension to hummingbird-flower relationships. *Oecologia*, 105(4), 517-523.
- Temeles, E. J., Goldman, R. S., & Kudla, A. U. (2005). Foraging and territory economics of sexually dimorphic purple-throated caribs (*Eulampis jugularis*) on three *Heliconia* morphs. *The Auk*, 122(1), 187-204.
- Temeles, E. J., Koulouris, C. R., Sander, S. E., & Kress, W. J. (2009). Effect of flower shape and size on foraging performance and trade-offs in a tropical hummingbird. *Ecology*, 90(5), 1147-1161.
- Temeles, E. J., & Kress, W. J. (2003). Adaptation in a plant-hummingbird association. *Science*, 300(5619), 630-633.
- Temeles, E. J., Miller, J. S., & Rifkin, J. L. (2010). Evolution of sexual dimorphism in bill size and shape of hermit hummingbirds (Phaethornithinae): a role for ecological causation. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365(1543), 1053-1063.
- Temeles, E. J., Pan, I. L., Brennan, J. L., & Horwitt, J. N. (2000). Evidence for ecological causation of sexual dimorphism in a hummingbird. *Science*, 289(5478), 441-443.
- Temeles, E. J., Rah, Y. J., Andicoechea, J., Byanova, K. L., Giller, G. S., Stolk, S. B., & Kress, W. J. (2013). Pollinator-mediated selection in a specialized hummingbird–*Heliconia* system in the Eastern Caribbean. *Journal of evolutionary biology*, 26(2), 347-356.
- Thébaud, E., & Fontaine, C. (2010). Stability of ecological communities and the architecture of mutualistic and trophic networks. *Science*, 329(5993), 853-856.
- Theodorou, P., Albig, K., Radzevičiūtė, R., Settele, J., Schweiger, O., Murray, T. E., & Paxton, R. J. (2017). The structure of flower visitor networks in relation to pollination across an agricultural to urban gradient. *Functional Ecology*, 31(4), 838-847.
- Theodorou, P., Baltz, L. M., Paxton, R. J., & Soro, A. (2020). Urbanisation is associated with shifts in bumblebee body size, with cascading effects on pollination. *Evolutionary Applications*.
- Theodorou, P., Radzevičiūtė, R., Settele, J., Schweiger, O., Murray, T. E., & Paxton, R. J. (2016). Pollination services enhanced with urbanization despite increasing pollinator parasitism. *Proceedings of the Royal Society B: Biological Sciences*, 283(1833), 20160561.
- Thompson, J. N. (1994). *The coevolutionary process*: University of Chicago Press.
- Thomson, J. D., & Goodell, K. (2001). Pollen removal and deposition by honeybee and bumblebee visitors to apple and almond flowers. *Journal of Applied ecology*, 38(5), 1032-1044.

- Travers, S. E., Temeles, E. J., & Pan, I. (2003). The relationship between nectar spur curvature in jewelweed (*Impatiens capensis*) and pollen removal by hummingbird pollinators. *Canadian Journal of Botany*, 81(2), 164-170.
- Traveset, A., Tur, C., Trøjelsgaard, K., Heleno, R., Castro-Urgal, R., & Olesen, J. M. (2015). Global patterns of mainland and insular pollination networks. *Global Ecology and Biogeography*.
- Turgeon, G., Vander Wal, E., Massé, A., & Pelletier, F. (2015). Born to be wild? Response of an urban exploiter to human-modified environment and fluctuating weather conditions. *Canadian journal of zoology*, 93(4), 315-322.
- Ulm, K. (1990). Simple method to calculate the confidence interval of a standardized mortality ratio (SMR). *American journal of epidemiology*, 131(2), 373-375.
- Ushimaru, A., Kobayashi, A., & Dohzono, I. (2014). Does urbanization promote floral diversification? Implications from changes in herkogamy with pollinator availability in an urban-rural area. *The American Naturalist*, 184(2), 258-267.
- van de Kerke, S. J., van Engelenhoven, T., van Es, A. L., Schat, L., van Son, L. M., Vink, S., Hemerik, L., van Velzen, R., Schranz, M. E., & Bakker, F. T. (2020). Capturing variation in floral shape: a virtual3D based morphospace for *Pelargonium*. *PeerJ*, 8, e8823.
- Van der Niet, T., Pirie, M. D., Shuttleworth, A., Johnson, S. D., & Midgley, J. J. (2014). Do pollinator distributions underlie the evolution of pollination ecotypes in the Cape shrub *Erica plukenetii*? *Annals of Botany*, 113(2), 301-316.
- Vandelook, F., Janssens, S., Gijbels, P., Fischer, E., Van Den Ende, W., Honnay, O., & Abrahamczyk, S. (2019). Nectar traits differ between pollination syndromes in Balsaminaceae. *Annals of Botany*, 124(2), 269-279.
- Verboven, H. A., Aertsen, W., Brys, R., & Hermy, M. (2014). Pollination and seed set of an obligatory outcrossing plant in an urban–peri-urban gradient. *Perspectives in Plant Ecology, Evolution and Systematics*, 16(3), 121-131.
- Verboven, H. A., Uyttenbroeck, R., Brys, R., & Hermy, M. (2014). Different responses of bees and hoverflies to land use in an urban–rural gradient show the importance of the nature of the rural land use. *Landscape and Urban Planning*, 126, 31-41.
- Walker, J. S., Grimm, N. B., Briggs, J. M., Gries, C., & Dugan, L. (2009). Effects of urbanization on plant species diversity in central Arizona. *Frontiers in Ecology and the Environment*, 7(9), 465-470.
- Waller, D. M. (1980). Environmental determinants of outcrossing in *Impatiens capensis* (Balsaminaceae). *Evolution*, 747-761.
- Wang, Q., Li, Y., Pu, X., Zhu, L., Tang, Z., & Liu, Q. (2013). Pollinators and nectar robbers cause directional selection for large spur circle in *Impatiens oxyanthera* (Balsaminaceae). *Plant Systematics and Evolution*, 299(7), 1263-1274.
- War, A. R., Taggar, G. K., Hussain, B., Taggar, M. S., Nair, R. M., & Sharma, H. C. (2018). Plant defence against herbivory and insect adaptations. *AoB Plants*, 10(4), ply037.
- Waser, N. M. (2006). *Plant-pollinator interactions: from specialization to generalization*: University of Chicago Press.

- Waser, N. M., Chittka, L., Price, M. V., Williams, N. M., & Ollerton, J. (1996). Generalization in pollination systems, and why it matters. *Ecology*, 77(4), 1043-1060.
- Wenzel, A., Grass, I., Belavadi, V. V., & Tschardt, T. (2020). How urbanization is driving pollinator diversity and pollination—A systematic review. *Biological Conservation*, 108321.
- Wiley, J. W., & Wunderle, J. M. (1993). The effects of hurricanes on birds, with special reference to Caribbean islands. *Bird Conservation International*, 3(4), 319-349.
- Willig, M. R., Presley, S. J., Bloch, C. P., & Genoways, H. H. (2010). Macroecology of Caribbean bats: effects of area, elevation, latitude, and hurricane-induced disturbance. In C. University of Chicago Press, IL (Ed.), *Island bats: evolution, ecology, and conservation*. (pp. 216-264).
- Wray, J. C., & Elle, E. (2015). Flowering phenology and nesting resources influence pollinator community composition in a fragmented ecosystem. *Landscape Ecology*, 30(2), 261-272.
- Young, H. J. (2008). Selection on spur shape in *Impatiens capensis*. *Oecologia*, 156(3), 535-543.
- Young, T. P., Stanton, M. L., & Christian, C. E. (2003). Effects of natural and simulated herbivory on spine lengths of *Acacia drepanolobium* in Kenya. *Oikos*, 101(1), 171-179.
- Zych, M. (2007). On flower visitors and true pollinators: The case of protandrous *Heracleum sphondylium* L.(Apiaceae). *Plant Systematics and Evolution*, 263(3-4), 159-179.

Annexes

Matériel supplémentaire Chapitre 2

Table S1. Table of the pictures used for the measurement of the length on <http://gesneriads.ua.edu/image-library/> (Clark), New York Herbarium (NY) and at the Montreal Botanical Garden on herbarium specimens or live specimens (Joly).

species	Source	ID picture	Top length (mm)	Bottom length (mm)
Gesneria acaulis	Clark	jlc_11303_02	43,7	37,5
Gesneria acaulis	Clark	jlc_11303_02	44,4	37,5
Gesneria citrina	NY	citrina-v-291-01113746	19,2	15,2
Gesneria citrina	NY	citrina-v-291-01113746	22,1	16,5
Gesneria citrina	NY	citrina-v-291-01159467	14,7	12,7
Gesneria cubensis	NY	cubensis-01401480	25,6	22,1
Gesneria cubensis	NY	cubensis-01401480	27,9	23,1
Gesneria cubensis	NY	cubensis-01401482	17,8	15,7
Gesneria cubensis	NY	cubensis-01401482	18,5	15,4
Gesneria cubensis	NY	cubensis-01401486	30,9	23,3
Gesneria cuneifolia	Clark	dunn_1	21,6	21,1
Gesneria cuneifolia	Clark	dunn_1	21,1	20,4
Gesneria cuneifolia	Clark	dunn_1	19,7	18,2
Gesneria cuneifolia	Clark	GES_cuneifolia_G869_G857_G763_1	20,6	20,0
Gesneria cuneifolia	Clark	GES_cuneifolia_G869_G857_G763_1	20,2	17,6
Gesneria cuneifolia	Clark	GES_cuneifolia_G869_G857_G763_1	23,5	21,8
Gesneria pedicellaris	Clark	GES_pedicellaris_pauciflora_sacatilis_1	29,0	26,1
Gesneria pedicellaris	Clark	jlc_11328_06	28,8	26,6
Gesneria pulverulenta	Clark	GES_pulverulenta_G1034_1	14,2	12,7
Gesneria pulverulenta	Clark	GES_pulverulenta_G1034_1	14,4	13,6
Gesneria pulverulenta	Clark	GES_pulverulenta_G1034_1	15,0	13,6

Gesneria purpurascens	Clark	jlc_10564_027	30,7	27,5
Gesneria purpurascens	Clark	JLC_12769_026	38,9	33,4
Gesneria purpurascens	Clark	JLC_12769_061	39,8	35,1
Gesneria purpurascens	Clark	JLC_15963_11	27,0	23,1
Gesneria quisqueyana	Clark	apr_72r9_11	19,9	14,2
Gesneria reticulata	Clark	JLC_14490_56	16,1	13,8
Gesneria reticulata	Clark	JLC_15967_029	20,2	18,5
Gesneria reticulata	Clark	jlc_12780_008	20,5	18,7
Gesneria sintenisii	Joly	None	22,0	14,0
Gesneria viridiflora	Clark	jlc_10509_101	17,9	14,4
Gesneria viridiflora	Clark	JLC_10509_92	21,7	12,8
Gesneria viridiflora	Clark	JLC_10524_19	18,1	11,1
Gesneria viridiflora	Clark	JLC_10552_19	22,9	12,4
Gesneria viridiflora	Clark	jlc_10554_20	19,7	11,6
Gesneria viridiflora	Clark	JLC_10561_32	14,2	8,6
Gesneria viridiflora	Clark	JLC_12797_45	18,1	7,0
Gesneria viridiflora	Clark	JLC_15984_130	23,5	7,5
Gesneria viridiflora	Clark	JLC_15984_138	17,4	10,6
Gesneria viridiflora	Clark	JLC_15984_248	16,6	9,0
Rhytidophyllum auriculatum	Joly	auriculatum_8892015A	17,8	8,2
Rhytidophyllum auriculatum	Joly	auriculatum_93711971	18,9	8,6
Rhytidophyllum auriculatum	Clark	JLC_14319_42	15,4	11,2
Rhytidophyllum auriculatum	Clark	JLC_14387_59	18,8	11,5
Rhytidophyllum auriculatum	Clark	JLC_14499_10	19,1	12,1
Rhytidophyllum auriculatum	Clark	JLC_14523_033	16,7	11,1
Rhytidophyllum auriculatum	Joly	None	17,0	11,0
Rhytidophyllum berterioanum	NY	berterioanum-01401909	16,6	14,3

Rhytidophyllum berteroanum	NY	berteroanum-01401921	16,6	15,6
Rhytidophyllum berteroanum	NY	berteroanum-01401921	13,9	12,3
Rhytidophyllum berteroanum	NY	berteroanum-01401923	14,4	13,5
Rhytidophyllum exsertum	Joly	exsertum_1121991	19,4	12,7
Rhytidophyllum exsertum	Joly	exsertum_10732010A	19,7	9,7
Rhytidophyllum exsertum	Clark	JLC_10508_13	22,1	13,5
Rhytidophyllum exsertum	Clark	JLC_10538_26	19,7	11,8
Rhytidophyllum exsertum	Clark	JLC_10546_20	18,6	12,6
Rhytidophyllum exsertum	Clark	JLC_10551_03	19,6	12,4
Rhytidophyllum exsertum	Clark	JLC_10571_07	19,7	12,4
Rhytidophyllum exsertum	Clark	JLC_10585_14	16,4	11,1
Rhytidophyllum exsertum	Clark	JLC_12787_28	17,6	12,2
Rhytidophyllum exsertum	Clark	JLC_14559_17	16,3	12,1
Rhytidophyllum exsertum	Clark	JLC_14559_19	17,3	11,4
Rhytidophyllum exsertum	Clark	JLC_14577_33	19,2	11,0
Rhytidophyllum exsertum	Clark	JLC_14594_36	16,8	11,6
Rhytidophyllum exsertum	Joly	None	20,0	12,0
Rhytidophyllum grandiflorum	Clark	apr72r9_8	20,9	14,4
Rhytidophyllum leucomallon	Clark	JLC_14338_031	18,0	14,2
Rhytidophyllum leucomallon	Clark	JLC_14497_31	23,3	12,0
Rhytidophyllum leucomallon	Clark	JLC_14498_16	18,1	10,7
Rhytidophyllum leucomallon	Joly	None	17,0	11,0

Rhytidophyllum minus	Clark	JLC_10547_17	16,4	10,7
Rhytidophyllum minus	Clark	JLC_10549_14	11,4	6,6
Rhytidophyllum minus	Clark	JLC_15982_053	14,9	12,2
Rhytidophyllum minus	Clark	jlc_10500_31	16,5	13,3
Rhytidophyllum vernicosum	Joly	vernicosum_12671966B	20,2	7,6
Rhytidophyllum vernicosum	Joly	None	15,0	10,0

Table S2. Photo credits of the all the pictures used to do the morphometric measures of the beak of the hummingbirds. For each species the scientific name, number of pictures collected, the sex and the source of the pictures are indicated.

Scientific name	Number of pictures	Sex	Sources
<i>Archilochus colubris</i>	7	male & female	eol
<i>Cyanophaia bicolor</i>	6	male & female	Sharp C., OpWall Dominica
<i>Mellisuga helenae</i>	6	male & female	Rodriguez R.
<i>Anthracothonax viridis</i>	6	male & female	Odeide R., Burgos E., Rodridriguez R.
<i>Anthracothonax mango</i>	10	ind	Snyder K., Rodriguez R., Sharp C., Dean G.
<i>Eulampis jugularis</i>	6	ind	Sharp C., Philippe_Boissel
<i>Chlorostilbon maugaeus</i>	8	male & female	Rodriguez R., Gonzalez J. & Collazo F., Plaza G., Archilla A., Guris P., Engie PR
<i>Chlorostilbon swainsonii</i>	6	male & female	Dax Roman, Pericles Brea
<i>Trochilus polytmus</i>	10	male & female	Snyder K., Jpnes P., Sharp C., Dean G. & Y., Rodriguez R.
<i>Eulampis holosericeus</i>	8	male	Burgos E.
<i>Orthorhyncus cristatus</i>	7	male & female	Sharp C., Burgos E.
<i>Chlorostilbon ricardii</i>	9	male & female	Wilson R., Sharp C., Rodriguez Carreon C.
<i>Mellisuga minima</i>	7	ind	Snyder K.
<i>Anthracothonax dominicus</i>	7	male & female	Armenteros G., Perez H., Dax Roman, Rodriguez R., Armanteros G.

Table S3. Result of the different linear regressions testing the relationship between the bottom length of the corolla and the beak shape of the pollinator when considering specialist and generalist plant species.

	Variable(s) tested	Estimate	P-value	R²
Corolla length (bottom)	beak length	0.779	0.051	0.177
	beak angle	0.215	0.202	0.080
	PC1+PC2+PC3			0.249
	PC1	-32.813	0.702	
	PC2	526.863	0.093	
	PC3	-163.973	0.400	

Table S4. Results of the different regressions done for the bottom corolla length, on the hummingbird specialists species.

	Variable(s) tested	Estimate	P-value	R²
Corolla length (bottom)	beak length	0.773	0.115	0.211
	beak angle	0.381	0.077	0.258
	PC1+PC2+PC3			0.603
	PC1 PC2 PC3	43.508 598.700 -641.243	0.630 0.051 0.025	

Table S5. Results of the different regressions done for the bottom corolla length, on the generalist species.

	Variable(s) tested	Estimate	P-value	R²
Corolla length (bottom)	beak length	-0.022	0.925	0.001
	beak angle	-0.043	0.548	0.047
	PC1+PC2+PC3			0.107
	PC1	-4.567	0.924	
	PC2	-27.759	0.904	
	PC3	52.155	0.624	

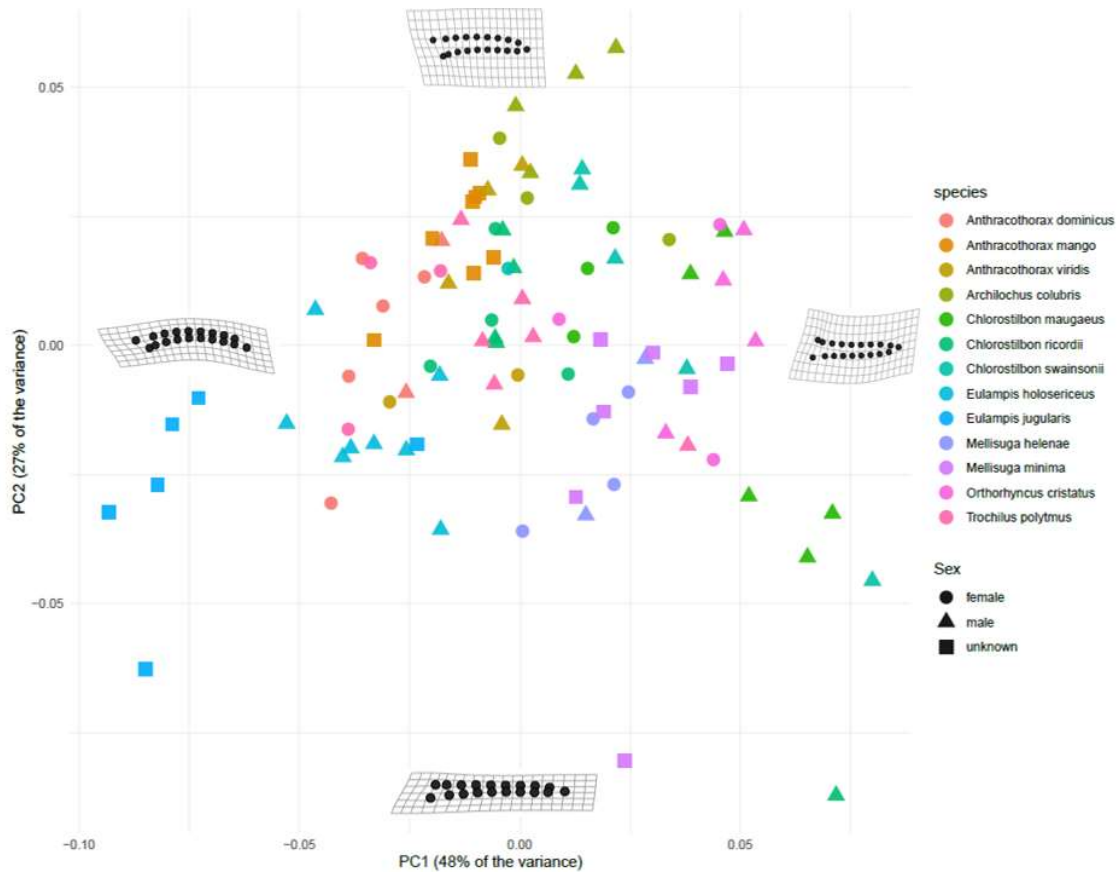


Figure S1. PCA on the beak shape of the hummingbirds showing the sex of the individuals. Females are represented by a triangle, males by a round, and unknown by a square. Thin-plate spline deformation grids show beak shape variation among the principal components, plus or minus 2 standard deviation from the mean shape.

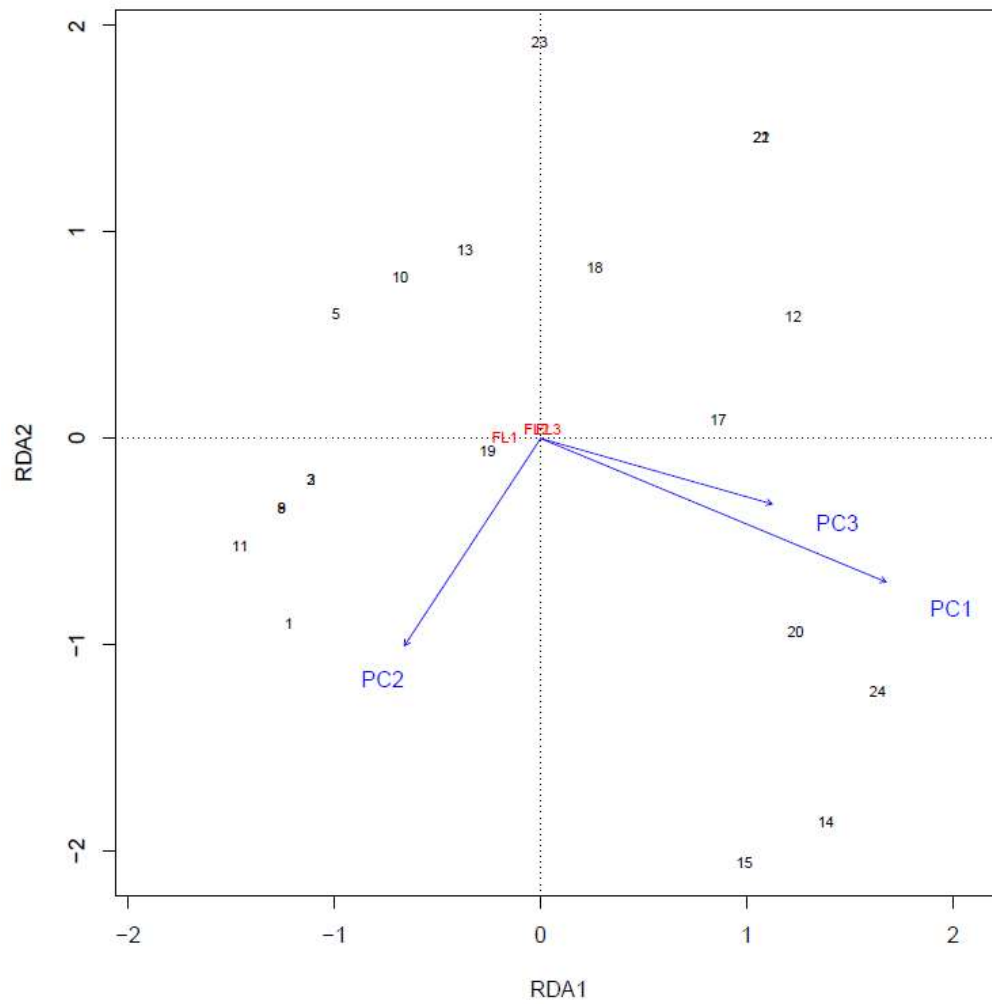


Figure S2. Multivariate redundancy analysis (RDA) testing the effect of beak shape (PC1, PC2, PC3) on floral shape (FL1, FL2, FL3) for generalist and specialist plant species. The plot was produced using a scaling of 2 in which the angle between vector reflect linear correlation.

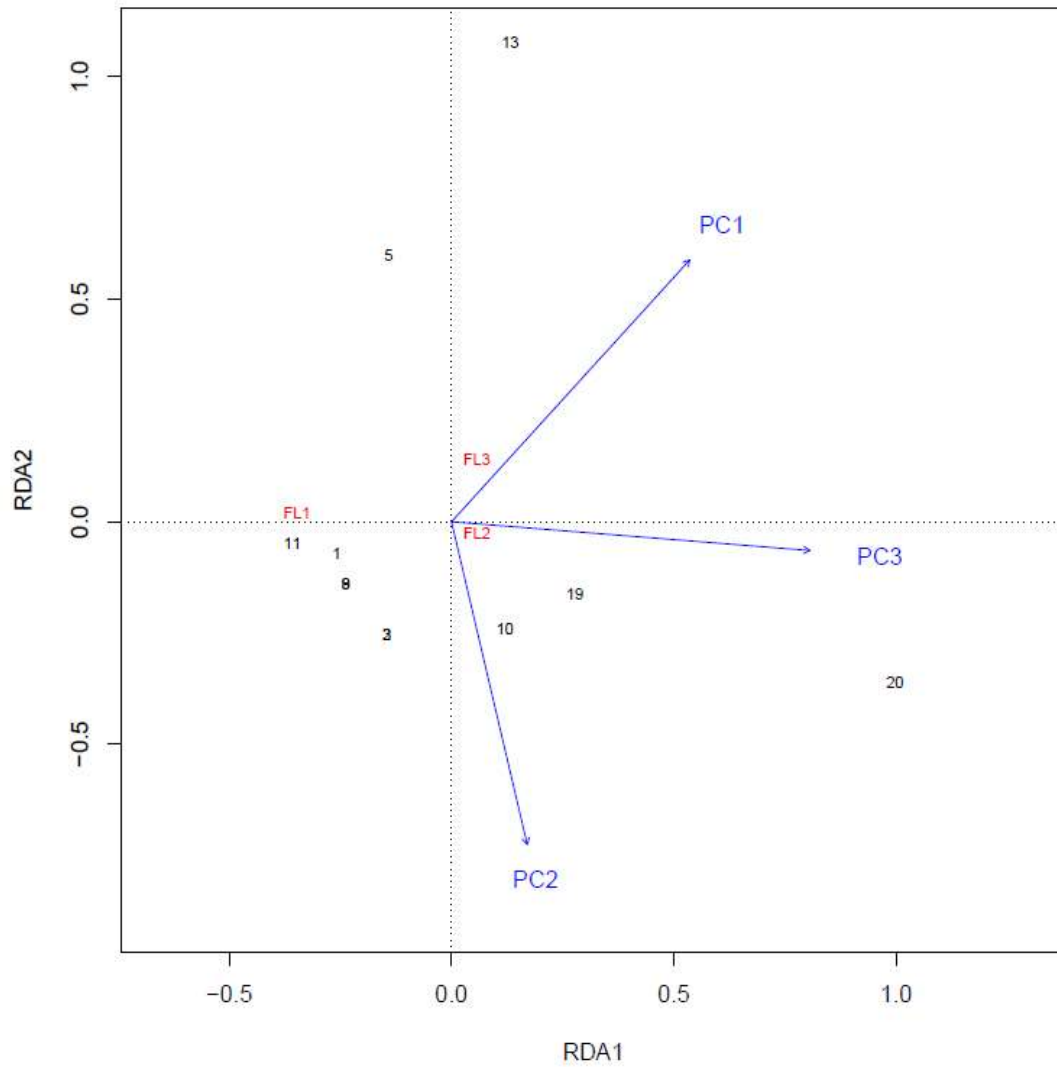


Figure S3. Multivariate redundancy analysis (RDA) testing the effect of beak shape (PC1, PC2, PC3) on floral shape (FL1, FL2, FL3) for specialist plant species. The plot was produced using a scaling of 2 in which the angle between vector reflect linear correlation.

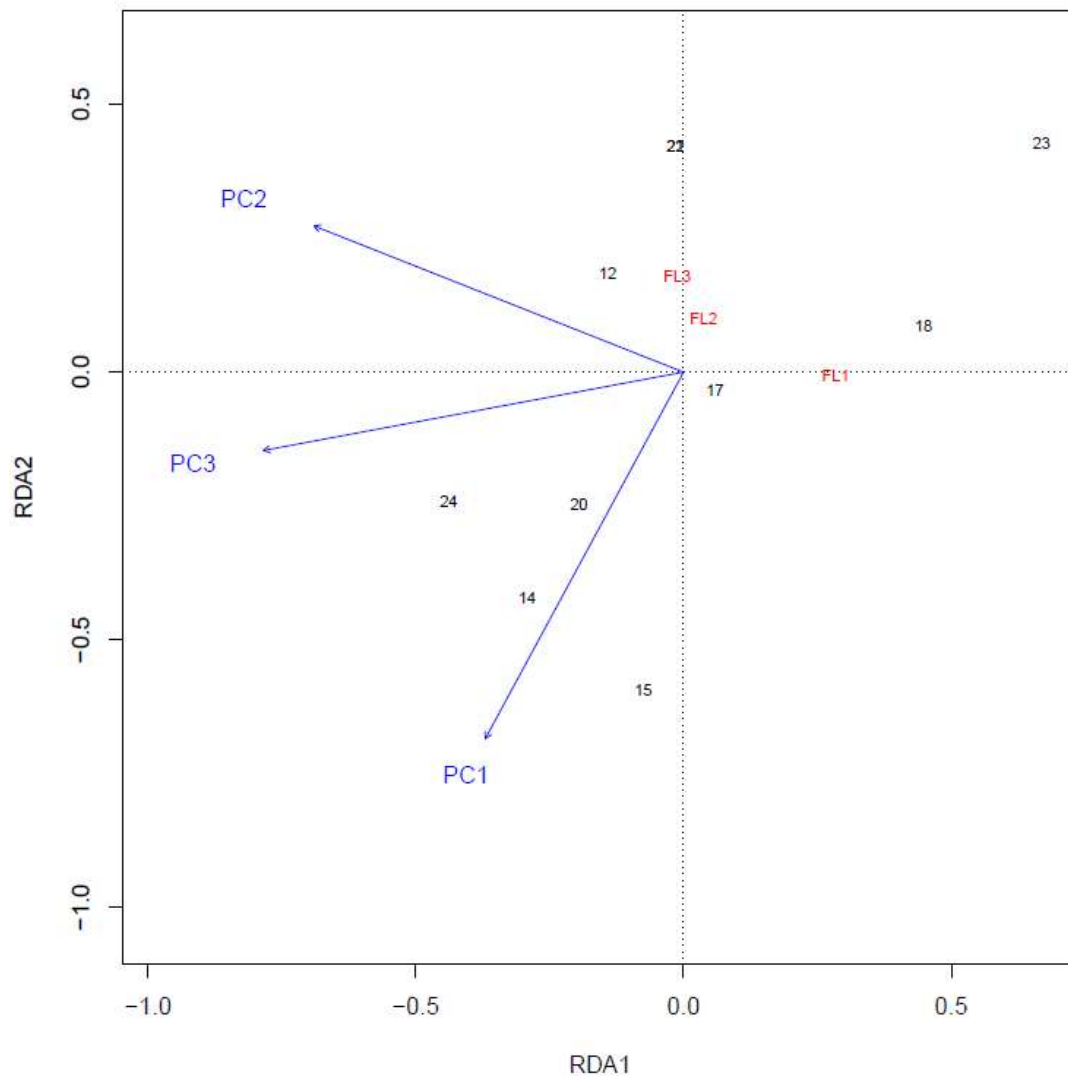


Figure S4. Multivariate redundancy analysis (RDA) testing the effect of beak shape (PC1, PC2, PC3) on floral shape (FL1, FL2, FL3) for generalist plant species. The plot was produced using a scaling of 2 in which the angle between vector reflect linear correlation.

Matériel supplémentaire Chapitre 3

Date	Start time	Weather	Habitat	Population name	Species	Number flowers observed	Observation period (hours)	Number of flower pollinised	Time of pollinations	Animal species observed
01/19/2018	7PM	fog	riparian	Ecole Formont	R. bicolor	10	2	0	-	M. redmani
01/20/2018	8:10AM	clear weather	riparian	Ecole Formont	R. bicolor	4	1	1	08:20PM	Bee
01/20/2018	10AM	clear weather	hill	Hauts Formont	R. bicolor	10	1	0	-	-
01/20/2018	12PM	clear weather	riparian	Ravin Formont	R. bicolor	10	1	0	-	-
01/20/2018	6:30PM	wind	riparian	Ecole Formont	R. bicolor	3	1	0	-	-
01/20/2018	9:45PM	wind	riparian	Ecole Formont	R. bicolor	3	1	1	-	M. redmani
01/21/2018	5:45PM	wind	hill	Bois Formont	R. bicolor	12	2.5	0	-	M. redmani
01/22/2018	7:30AM	clear weather	canyon	Fonblé	R. bicolor	10	3	0	-	-
01/22/2018	11AM	clear weather	field border	Hauts Formont	R. bicolor	10	1	3	-	Bee
01/22/2018	2PM	clear weather	hill	Mont cavalier	R. bicolor	10	2	0	-	-
01/22/2018	7PM	clear weather	riparian	Ecole Formont	R. bicolor	13	3	1	9PM	M. redmani
01/23/2018	6:45PM	fog	riparian	Ecole Formont	R. bicolor	12	3	9 visits on non receptive flowers	8:20 - 9:55PM	M. redmani
01/24/2018	8AM	clear weather	hill	Bois Formont parc	R. bicolor	10	2	0	-	M. minima
01/24/2018	3:30PM	clear weather	hill	Bois Formont parc	R. bicolor	10	1	0	-	M. minima
01/24/2018	8PM	clear weather	riparian	Ecole Formont	R. bicolor	13	1	5	8PM - 8:55PM	M. redmani
01/25/2018	6:30AM	clear weather	riparian	Ravin Formont	R. bicolor	10	1	0	-	-

01/25/2018	8AM	clear weather	hill	Formont	R. bicolor	10	2	0	-	-
01/25/2018	1:30PM	fog	hill	Mont cavalier	R. bicolor	10	1.5	0	-	-
01/25/2018	7:30PM	cloudy	riparian	Ecole Formont	R. bicolor	17	2	4	8:05 - 9:12PM (M. redmani)	M. redmani
01/26/2018	6:30AM	clear weather	riparian	Ravine	R. bicolor	10	3	0	-	-
01/27/2018	6:30AM	fog	hill	Mont cavalier, Bois Formont	R. bicolor	10	3.5	0	-	-
01/27/2018	7:30PM	fog	riparian	Ecole Formont	R. bicolor	12	2	1	-	M. redmani, moth
01/28/2018	7:30PM	wind, rain, fog	riparian	Ecole Formont	R. bicolor	10	0.5	0	-	-

Matériel supplémentaire Chapitre 4

Table S1. Visitation rate (i.e. visit by flower by hour) for each visitor species observed at each sites. The visitation rate of non-pollinators (identified with an *) was not used in the analysis.

Species	Common name	SU-RSF	SU-UFO	SU-RB	N-RDN	N-SBL	N-OKA
<i>Bombus sp.</i> (<i>B. Impatiens</i> and/or <i>B. vagans</i>)	Common eastern bumblebee and/or half-black bumblebee	0.551	0.615	0.229	0.336	0.237	2.421
<i>Apis mellifera</i>	Western honeybee	0.494	0.019	0.046	0.024	0.243	0.020
<i>Archilochus colubris</i>	Ruby-throated hummingbird	0	0	0.036	0	0	0.109
<i>Bombus ternarius</i>	Orange belted bumblebee	0	0	0	0	0.012	0
<i>Vespa sp.</i>	Wasp	0	0.012	0.026	0.005	0.004	0.008
<i>Anthidium florentinum</i>	European wool carder bee	0.04347826	0	0	0	0	0
<i>Ocyrtamus fascipennis</i> *	Syrphid fly	0	0	0	0	0	0.013
<i>Syrphus sp.</i> *	Hoverfly	0	0	0	0.008	0	0
<i>Rhingia nasica</i> *	Syrphid fly	0	0.016	0.009	0.04	0.008	0.013
<i>Toxomerus geminatus</i> *	Flower fly	0	0	0	0.007	0.003	0
<i>Augochlorella aurata</i> *	Sweat bee	0.017	0	0.017	0.008	0	0.004
<i>Andrena sp.</i> *	Mining bee	0	0.012	0.028	0.016	0	0
<i>Xylocopa sp.</i> *	Carpenter bee	0	0	0.014	0	0	0
<i>Sceliphron caementarium</i> *	Mud dauber	0	0	0.031	0	0	0

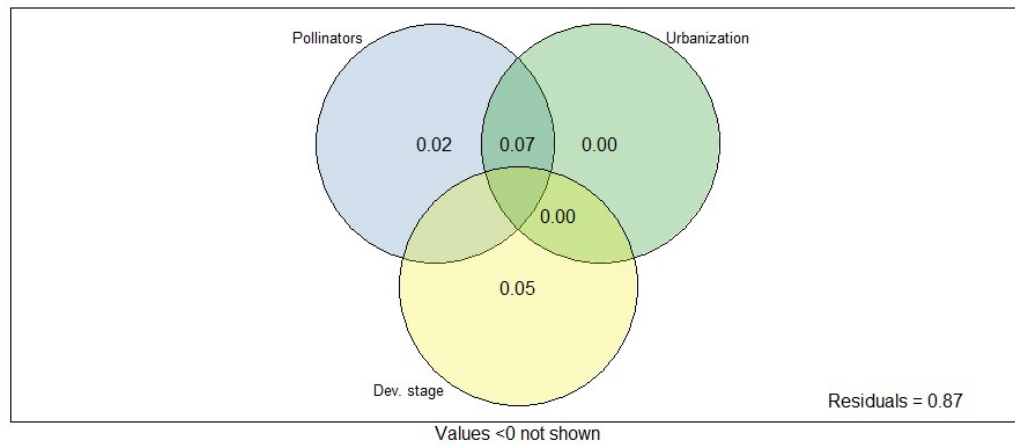


Figure S1. Venn diagram of the floral shape variance partitioning between the pollinator visitation rates, the urbanisation and the developmental stage of flowers.